LOWER DRUG COSTS NOW: EXPANDING ACCESS TO AFFORDABLE HEALTH CARE

HEARING

BEFORE THE

SUBCOMMITTEE ON HEALTH, EMPLOYMENT, LABOR, AND PENSIONS

OF THE

COMMITTEE ON EDUCATION AND LABOR U.S. HOUSE OF REPRESENTATIVES

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LOWER DRUG COSTS NOW: EXPANDING ACCESS TO AFFORDABLE HEALTH CARE

Wednesday, May 05, 2021

HOUSE OF REPRESENTATIVES,
SUBCOMMITTEE ON HEALTH, EMPLOYMENT,
LABOR, AND PENSIONS,
COMMITTEE ON EDUCATION AND LABOR,
Washington, DC.

The Subcommittee met, pursuant to notice, at 12 p.m., via Zoom, Hon. Mark DeSaulnier (Chairman of the Subcommittee) presiding.

Present: Representatives DeSaulnier, Courtney, Norcross, Wild, McBath, Stevens, Levin, Mrvan, Scott (*ex officio*), Allen, Wilson, Walberg, Harshbarger, Miller, Fitzgerald, Foxx (*ex officio*), and Spartz.

Staff present: Ilana Brunner, General Counsel; David Dailey, Counsel to the Chairman; Ijeoma Egekeze, Professional Staff; Daniel Foster, Health and Labor Counsel; Rashage Green, Director of Education Policy; Eli Hovland, Policy Associate; Carrie Hughes, Director of Health and Human Services; Ariel Jona, Policy Associate; Max Moore, Staff Assistant; Yonatan Moskowitz, Oversight Counsel—Labor; Mariah Mowbray, Clerk/Special Assistant to the Staff Director; Kayla Pennebecker, Staff Assistant; Véronique Pluviose, Staff Director; Banyon Vassar, Deputy Director of Information Technology; Everett Winnick, System Administrator; Cyrus Artz, Minority Staff Director; Courtney Butcher, Minority Director of Member Services and Coalitions; Rob Green, Minority Director of Workforce Policy; Taylor Hittle, Minority Professional Staff Member; Georgie Littlefair, Minority Legislative Assistant; John Martin, Minority, Minority Workplace Policy Counsel; Hannah Matesic, Minority Director of Operations; Audra McGeorge, Minority Communications Director; Carlton Norwood, Minority Press Secretary; Ben Ridder, Minority Professional Staff Member.

Chairman DESAULNIER. The Subcommittee on Health, Employment, Labor, and Pensions will come to order. Welcome everyone. I note that a quorum is present. I note for the Subcommittee that Mrs. Spartz of Indiana is permitted to participate in today's hearing with the understanding that her questions will come only after Members of the HELP Subcommittee on both sides of the aisle who are present have had an opportunity to question the witnesses.

The Subcommittee is meeting today to hear testimony on "Lower Drug Costs Now: Expanding Access to Affordable Health Care." This is an entirely remote hearing. All microphones will be kept muted as a general rule to avoid unnecessary background noise. Members and witnesses will be responsible for unmuting them-

selves when they are recognized by the Chair to speak, or when they wish to seek recognition.

I also ask that Members please identify themselves before they speak. Members should keep their cameras on while in the proceeding. Members shall be considered present in the proceeding when they are visible on camera, and they shall be considered not present when they are not visible on camera.

The only exception to this is if they are experiencing technical difficulty, and inform Committee staff of such difficulty. If any Member experiences technical difficulties during the hearing you should stay connected on the platform, make sure you are muted and use your phone to immediately call a Committee system administration whose number has been provided in advance.

Should the Chair experience technical difficulty, Mr. Courtney, the distinguished gentleman from Connecticut, or another majority Member is hereby authorized to assume the gavel in the Chair's absence. Again this is an entirely remote hearing and as such the Committee's hearing room is officially closed.

Members who choose to sit with their individual devices in the hearing room must wear headphones to avoid feedback, echoes and distortion resulting from more than one person on a software platform sitting in the same room.

Members are also expected to adhere to social distancing and safe health care guidelines, including the use of masks, hand sanitizer and wiping down their areas both before and after their presence in the hearing room.

In order to ensure that the Committee's five-minute rule is adhered to, staff will be keeping track of time using the Committee's field timer. The field timer will appear in its own thumbnail picture and will be named 001__timer. There will be no one-minute remaining time warning.

The field timer will show a blinking light when your time is up. Members and witnesses are asked to wrap up promptly when their time has experience. While a roll call is not necessary to establish a quorum in official proceedings conducted remotely, or with remote participation, the Committee has made it a practice whenever there is an official proceeding with remote participants for the Clerk to call the role, and help make clear who is present at the start of the proceeding.

Members should say their name before announcing they are present. This helps the Clerk and also helps those who are watching the platform and the livestream who may experience a few seconds delay. At this time I will ask the Clerk to call the role.

The CLERK. Chair DeSaulnier?

Chairman DESAULNIER. Here.

The CLERK. Mr. Courtney?

Mr. COURTNEY. I'm here.

The CLERK. Mr. Norcross?

Mr. NORCROSS. Here.

The CLERK. Mr. Morelle?

[No response]

The CLERK. Ms. Wild?

[No response]

The CLERK. Mrs. McBath?

[No response]
The CLERK. Ms. Stevens?
[No response]
The CLERK. Mr. Levin?
[No response]
The CLERK. Mr. Mrvan?
[No response]
The CLERK. Mr. Scott?
Mr. SCOTT. Mr. Scott is present.
The CLERK. Ranking Member Allen?
Mr. ALLEN. Allen present sorry about the mute button.
The CLERK. Mr. Wilson?
Mr. WILSON. Present,
The CLERK. Mr. Walberg?
Mr. WALBERG. Walberg present.
The CLERK. Mr. Banks?
[No response]
The CLERK. Mrs. Harshbarger?
Mrs. HARSHBARGER. Harshbarger present.
The CLERK. Mrs. Miller?
[No response]
The CLERK. Mrs. Miller could you repeat yourself? OK. Mr. Fitzgerald?
Mrs. MILLER. Can you hear me?
Mr. FITZGERALD. Yes I got you, I'm here.
Mrs. MILLER. Mrs. Miller I'm present.
The CLERK. Mrs. Miller I have you recorded thank you. And Mrs. Foxx?
Mrs. FOXX. Foxx is present.
The CLERK. Thank you. Chairman DeSaulnier that concludes the roll call.
Chairman DESAULNIER. Thank you.
Mrs. MCBATH. Chairman DeSaulnier I just wanted you to know I'm here, McBath is here, I'm present.
Chairman DESAULNIER. Thank you Ms. McBath.
Mrs. MCBATH. Thank you.
Chairman DESAULNIER. Welcome. Pursuant to Committee Rule 8(c) opening statements are limited to the Chair and the Ranking Member. This allows us to hear from witnesses sooner and provides all Members with adequate time to ask questions. I now recognize myself for the purpose of making an opening statement.

Welcome everyone to this important Subcommittee hearing. Good morning, at least good morning on the west coast. We are here today to discuss the cost of prescription medication and how lowering drug prices would support workers and strengthen the American economy.

It has been more than a year since the House first passed the Elijah E. Cummings Lower Drug Costs Now Act to reduce out of control participation in our costs. Yet on top of weathering the worst global health crisis in recent history, Americans are still paying far too much for the medication they need.

Robert from my district reached out to my office just recently when his monthly cost of his HIV medication that keeps him alive

went from $130 to $960 per month, and of course unfortunately Robert is not alone.

Each year the prices of hundreds of drugs increase faster than the rate of inflation. Today annual per capital spending on prescription drugs in the United States is more than $1,200. And the total out of pocket costs that consumers pay for drugs is more than $80 billion a year.

These unaffordable prices have significant real-world consequences. Even in 2008 three in 10 adults decided to forego their prescribed medication due to the prohibitive cost. This is particularly frustrating given that these are not the prices that consumers in the rest of the world pay.

Americans routinely pay three to four times, sometimes a dozen times more than what patients in other countries pay for the exact same drug. In fact, the cancer drug that keeps me alive that I take for my chronic lymphocytic leukemia that as I said is chronic, but is treatable thank goodness. My drug costs $500 per day. Ibrutinib.

I'm grateful obviously, for this drug, but I wonder why in Australia the same diagnosis provides the exact same prescription drug for less than $30 a month. $500 in the United States, less than $30 in Australia. But the high cost of drug prices doesn't hurt just consumers. It also costs our nations' businesses which sponsors the health coverage of approximately 150 million Americans.

These plans spent nearly $84 billion on drugs in 2016 alone. And this price tag is only expected to grow unless we act. Some of my colleagues may argue that high drug prices are needed to cover the cost of research and developing and deploying new drugs, yet we know that most drug companies spend more on marketing, sales, and overhead than they do on research and development.

No one, myself included obviously, wants to stop the wonderful innovation and deployment of life-saving drugs like the one I take. However, however we must consider an appropriate return on investment that incentivizes the private sector and continues to support the important development of drugs at the NIH with public tax dollars, and also with the Department of Defense and the Department of Defense in DARPA.

Profits for drug companies are among the highest of any sector. According to the GAO the average profit margin for a large drug company is between 50 and 20 percent after taxes. Compared to the average large company's margin in other sectors which is somewhere between 4 and 10 percent.

While drug companies focus on their bottom line, the vast majority of new life-saving drugs are developed thanks, as I said, to taxpayer funded medical research, like the important work happening today at the National Institute for Health. In fact, every drug that was approved by the FDA in 2010 through 2016 was developed at least in part with NIH supported research.

Investing in research is vital to developing new cures and technologies, but we need fair drug prices at the other end, so that consumers can actually realize these investments and save their lives and promote their health. That is why people across the political spectrum and across the country have made clear that they want Congress to reign in prescription drug prices.

The Lower Drug Costs Now Act takes bold steps to lower the cost of drugs and increase transparency. First, it finally allows the Secretary of Health and Human Services to negotiate directly with drug companies to get fair prices for those in Medicare.

Second, the bill makes the lower drug prices negotiated by Medicare available to consumers with private health coverage, including those covered with employer sponsored plans. This will not only cut costs for workers covered by employer provided health care it will cut costs for employees as well.

Third, the legislation caps negotiated drug prices to align with prices charged in similar countries, ensuring that Americans are no longer price gouged at the pharmacy counter. Fourth, the bill sets a new limit on out of pocket drug costs for Medicare beneficiaries and ends unfair annual price hikes by prescription drug companies for 8,000 drugs.

And, finally, the Lower Drug Costs Now Act takes advantage of the savings from negotiating lower drug prices by reinvesting that savings back into research to find new medical breakthroughs.

As President Biden reminded us in his address to Congress last week, we've talked about lowering the cost of prescription drugs for long enough. Democrats and Republicans. It's time to finally deliver on our promise to ensure that all Americans can get the medication they need to stay healthy and thrive.

I now recognize the distinguished Ranking Member for the purpose of making an opening statement.

[The statement of Chairman DeSaulnier follows:]

STATEMENT OF HON. MARK DESAULNIER, CHAIRMAN,
SUBCOMMITTEE ON HEALTH, EMPLOYMENT, LABOR, AND PENSIONS

We are here today to discuss the cost of prescription medication and how lowering drug prices would support workers and strengthen the American economy.

It has been more than a year since the House first passed the Elijah E. Cummings Lower Drug Costs Now Act to reduce out-of-control prescription drugs costs. Yet, on top of weathering the worst global health crisis in recent history, Americans are still paying far too much for the medication they need.

Robert from my district reached out to my office just recently when the monthly cost of his HIV medication that keeps him alive went from $130 to $960 a month. And, unfortunately, Robert is not alone.

Each year, the prices of hundreds of drugs increase faster than the rate of inflation. Today, annual per capita spending on prescription drugs in the United States is more than $1,200, and the total out-of-pocket costs that consumers pay for drugs is more than $80 billion a year.

These unaffordable prices have significant real-world consequences. Even in 2018, three in ten adults decided to forgo their prescribed medication due to the prohibitive cost.

This is particularly frustrating given that these are not the prices that consumers in the rest of the world pay. Americans routinely pay three or four times—sometimes a dozen times—more than what patients in other countries pay for the exact same drugs.

In fact, the cancer drug that keeps me alive, which I take for my chronic lymphocytic leukemia that is chronic, but is treatable—thank goodness—costs $500 a day. I am grateful, obviously, for this drug, but I wonder why, in Australia, the same diagnosis provides the exact same prescription drug for less than $30 a month. $500 in the United States; less than $30 in Australia.

But the high cost of drug prices doesn't hurt just consumers. It also costs our Nation's businesses, which sponsor the health coverage of approximately 150 million people. These plans spent nearly $84 billion on drugs in 2016, alone, and this price tag is only expected to grow unless we act.

Some of my colleagues may argue that high drug prices are needed to cover the cost of researching, developing, and deploying new drugs. Yet, we know that most

drug companies spend more on marketing, sales, and overhead than they do on research and development.

No one—myself included, obviously—wants to stop the wonderful innovation and deployment of lifesaving drugs, like the one I take. However, we must consider an appropriate return on investment that incentivizes the

private sector and continues to support the important development of drugs at the NIH with public taxpayer dollars and also in the Department of Defense and DARPA.

Profits for drug companies are among the highest of any sector. According to the GAO, the average profit margin for a large drug company is between 15 and 20 percent after taxes—compared to the average large company's margin in other sectors, which is somewhere between 4 and 10 percent.

While drug companies focus on their bottom line, the vast majority of new, lifesaving drugs are developed thanks—as I said—to taxpayer-funded medical research, like the important work happening today at the National Institutes for Health. In fact, every drug that was approved by the FDA from 2010 through 2016 was developed at least in part with NIH-supported research.

Investing in research is vital to developing new cures and technologies, but we need fair drug prices at the other end so that consumers can actually realize these investments and save their lives and promote their health.

That is why people across the political spectrum and across the country have made clear that they want Congress to rein in prescription drug prices.

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Finally, the Lower Drug Costs Now Act takes advantage of the savings from negotiating lower drug prices by reinvesting those savings back into research to find new medical breakthroughs.

As President Biden reminded us in his address to Congress last week, we've talked about lowering the cost of prescription drugs for long enough—Democrats and Republicans. It's time to finally deliver on our promise to ensure that all Americans can get the medication they need to stay healthy and thrive.

Mr. ALLEN. Thank you Mr. Chairman. When the Trump administration launched Operation Warp Speed in May 2020 they did so with a bold mission, to develop a COVID–19 vaccine by the end of the year. Seven months later healthcare workers lined up to receive their first dose of the Pfizer vaccine.

Today over 100 million adults are vaccinated against COVID–19. This lifesaving scientific technological and logistical feat demonstrates just how powerful, innovative, and effective the American healthcare system can be. Unfortunately, many Americans are facing skyrocketing healthcare costs because of dramatic increases and out of pocket costs of prescription drugs.

In 2018 patients paid a collective 61 billion in out of pocket drug costs. Fortunately, republicans are stepping up with solutions that work best for the people, without the heavy hand of government interference. H.R. 19, the Lower Cost More Cures Act introduced last month by republican Committee leaders is exact opposite of H.R. 3, Pelosi's big government power grab.

The Lower Costs More Cares Act will utilize the power of the free market to modernize our healthcare system, increase choice in transparency and lower costs. These are goals that both sides of the aisle should be able to rally behind and focus on. All the provisions in H.R. 19 are bipartisan, workable and ready to become law. We have the power to stabilize and ease the minds of Americans struggling to pay for needed medications.

Up until 2019 congressional efforts to lower drugs prices for the American people were a collaborative effort and bipartisan effort. That changed when Speaker Pelosi wrote the Democrats devastating Drug Pricing Plan H.R. 3 behind closed doors and without any republican input.

This year the democrats are doubling down on H.R. 3, radical legislation which would have a devastating affect on drug development and innovation in the United States. After the Council of Economic Advisers found that H.R. 3 would lead to 100 fewer drugs entering the marketplace, Republican Leader McCarthy said it best. "HR 3 is a step toward nationalizing the drug industry, and opening the door to one size fits all government control rationing of prescription drugs."

Once again democrats are pushing far left politics over policy by rushing through a harmful partisan bill. According to the non-partisan Professional Budget Office H.R. 3 would significantly decrease private investment in research and development.

Messenger RNA, the technology used to make the Pfizer and Moderna vaccines took billions of dollars of private investment, over three decades to develop. Had this bill been long before the pandemic's speed of development of Moderna and Pfizer vaccines, it would likely not have been possible.

Countries around the world are benefiting from this life-saving technology because the Senate refused to pass the democrat socialist healthcare scheme. You think that would give my democrat colleagues pause. Instead democrats are again trying to pass at partisan speed that would increase our reliance on Chinese medical manufacturing, reduce our capacity for innovation, and devalue people with disabilities and chronic illnesses.

I would ask the democrats in the U.S. House of Representatives to support Medicare for All, a government run single payer healthcare system that would cost 32 trillion dollars, and eliminate private insurance, including employer sponsored coverage which benefits 159 million Americans and is the jurisdiction of this Committee.

By contrast, 71 percent of Americans say they are satisfied with their employer sponsored healthcare coverage. Now democrats want to pass this harmful bill to move Americans in employer sponsored plans to Medicare, bringing us even closer to a socialist single payer system.

This should be common sense, but kicking Americans off their healthcare plan will not increase their access to affordable medical care. They will do the opposite. At the end of the day H.R. 3 would only lead to fewer treatments and cures, decrease competition in the marketplace, and increase reliance on Communist China.

Unfortunately, the hearing today is being held to promote this radical scheme, rather than promote partisan socialist policies such

as H.R. 3, I urge my colleagues to work together on finding a bipartisan solution to lowering drug costs, like the common sense provisions in H.R. 19.

I thank all the witnesses for joining us here today and I yield back the balance of my time.

[The statement of Ranking Member Allen follows:]

STATEMENT OF HON. RICK W. ALLEN, RANKING MEMBER, SUBCOMMITTEE ON HEALTH, EMPLOYMENT, LABOR, AND PENSIONS

When the Trump administration launched Operation Warp Speed in May 2020, they did so with a bold mission: develop a COVID–19 vaccine by the end of the year. Seven months later, health care workers lined up to receive their first dose of the Pfizer vaccine.

To date, over 100 million adults are vaccinated against COVID–19. This lifesaving scientific, technological, and logistical feat demonstrates just how powerful, innovative, and effective the American health care system can be.

Unfortunately, many Americans are facing skyrocketing health care costs because of dramatic increases in out-of-pocket costs of prescription drugs. In 2018, patients paid a collective $61 billion in out-of-pocket drug costs.

Fortunately, Republicans are stepping up with solutions that work best for the people, without the heavy hand of government interference. H.R. 19, the Lower Cost, More Cures Act, introduced last month by Republican committee leaders, is the exact opposite of H.R. 3, Pelosi's big government power grab. The Lower Cost, More Cures Act will utilize the power of the free market to modernize our health care system, increase choice and transparency, and lower costs. These are goals that both sides of the aisle should be able to rally behind.

All the provisions in H.R. 19 are bipartisan, workable, and ready to become law. We have the power to save lives and ease the minds of Americans struggling to pay for needed medications.

Up until 2019, congressional efforts to lower drug prices for the American people were a collaborative and bipartisan effort. That changed when Speaker Pelosi wrote the Democrats' devastating drug pricing plan, H.R. 3, behind closed doors and without any Republican input.

This year, the Democrats are doubling down on H.R. 3, radical legislation which would have a devastating effect on drug development and innovation in the United States. After the Council of Economic Advisors found that H.R. 3 would lead to 100 fewer drugs entering the marketplace, Republican Leader McCarthy said it best: H.R. 3 is 'a step toward nationalizing the drug industry and opening the door to a one-size-fits-all, government-controlled rationing of prescription drugs.

Once again, Democrats are pushing far-left politics over policy by rushing through a harmful, partisan bill. According to the nonpartisan Congressional Budget Office, H.R. 3 would significantly decrease private investment in research and development. Messenger RNA, the technology used to make the Pfizer and Moderna vaccines, took billions of dollars of private investment and over three decades to develop. Had this bill been law before the pandemic, the speedy development of the Moderna and Pfizer vaccines would likely not have been possible. Countries around the world are benefiting from this life-saving technology because the Senate refused to pass the Democrats' socialist health care scheme. You'd think that would give my Democrat colleagues pause.

Instead, Democrats are again trying to pass a partisan scheme that would increase our reliance on Chinese medical manufacturing, reduce our capacity for innovation, and devalue people with disabilities and chronic illnesses.

Over half of the Democrats in the U.S. House of Representatives support Medicare-for-All, a government-run, single-payer health care system that would cost $32 trillion and eliminate private insurance, including employer-sponsored coverage, which benefits 159 million Americans and is in the jurisdiction of this Committee. By contrast, 71 percent of Americans say they are satisfied with their employer-sponsored health care coverage. Now, Democrats want to pass this harmful bill to move Americans in employer-sponsored plans to Medicare, bringing us even closer to a socialist single-payer system.

This should be common sense, but kicking Americans off their health care plans will not increase their access to affordable medical care—it will do the opposite.

At the end of the day, H.R. 3 would only lead to fewer treatments and cures, decreased competition in the marketplace, and an increased reliance on Communist China.

Unfortunately, the hearing today is being held to promote this radical scheme. Rather than promote partisan socialist policies such as H.R. 3, I urge my colleagues to work together on finding a bipartisan solution to lowering drug costs, like the common-sense provisions included in H.R. 19.

Chairman DESAULNIER. Thank you Mr. Allen. And I will say my door is always open. Without objection all of the Members who wish to insert written statements into the record may do so by submitting them to the Committee Clerk electronically in Microsoft Word format by 5 p.m. on May 19, 2021.

I will now introduce the witnesses and then recognize them for their five minutes and then we'll go to questions. First Dr. Mariana Socal is an Associate Scientist at the Johns Hopkins University, Bloomberg School of Public Health. She is a medical doctor by training, and a researcher with expertise in prescription drug costs.

Next will be David Mitchell, he's Founder and President of Patients for Affordable Drugs. He is a cancer patient and an advocate for lower drug costs.

Dr. Douglas Holtz-Eakin is President of the American Action Forum, an economist by training. He is the former Director of the Congressional Budget Office.

Frederick Isasi is Executive Director of Families USA. He is a leading advocate for health care consumers on a wide range of issues. We appreciate the witnesses for participating today and very much look forward to your testimony. Let us remind the witnesses that we have read your statements, your written statements, and they will appear in full in the hearing record.

Pursuant to Committee Rule 8(d) and Committee practice, each of you is asked to limit your oral presentation to a five minute summary of your written statement. Before you begin your testimony please remember to unmute your microphone when you're recognized.

During your testimony staff will be keeping track of time and the timer will show a blinking light when time is up. Please be attentive to the time, wrap up when your time is over, and remute your microphone. If any of you experience a technical problem during your testimony, or later in the hearing, you should stay connected on the platform, make sure you are muted, and use your phone to immediately call a Committee system administrator, whose number was provided to you in advance.

We will let all witnesses make their presentations before we move to Members questions. When answering a question please remember to unmute your microphone. The witnesses are aware of their responsibility to provide accurate information to the Subcommittee and therefore we will proceed now with their testimony.

I first recognize Dr. Socal. Dr. Socal, the floor is yours.

STATEMENT OF DR. MARIANA SOCAL, MD, PH.D., MS, MPP, ASSOCIATE SCIENTIST, JOHNS HOPKINS BLOOMBERG SCHOOL OF PUBLIC HEALTH

Dr. SOCAL. Chairman DeSaulnier, Ranking Member Allen and Members of the Committee it is a great honor to be speaking with you today. The last few years I have received funding to work with

several large organizations that are attempting to control drug spending.

These include the Purchaser Business Group of Health, PBGH, and ERIC—The ERISA Industry Committee. I also received funding from Arnold Ventures. I've done extensive research examining the drug benefits that self-insured employers, from school districts to America's largest corporations offer to their workers.

Today about one-third of all Americans are covered by employer self-sponsored health plans. These self-insurance employers typically hire a pharmacy benefit manager, a PBM to negotiate drug prices on their behalf. The PBM negotiates with drug manufacturers buy offering to cover a drug more favorably in exchange for a lower price.

When the PBM can pick between multiple products the one that it will cover, then the market works. When a drug has no competition because there is no alternatives, then manufacturers will not lower their price, and the negotiation will fail. For drugs without competition the U.S. face three to four times higher prices than other countries.

For these drugs, a different type of negotiation is greatly needed in the approach adopted by H.R. 3 can offer a successful alternative. My colleagues and I examined the top spending drugs in Medicare Part D, and found that if the U.S. paid the average price across the countries that we studied, Medicare Part D alone could have saved about 73 billion dollars in 2018.

Employers savings would be similar. The drugs with the largest price differential compared to other countries are not the new drugs, but the drugs that have been in the U.S. market for a long period time. While drug prices in other countries tend to go down over time, drug prices in the U.S. continually increase.

Just this January, a record number of more than 800 drugs raised their U.S. prices. 99.9 percent of them were branded drugs, and most of them had also increased their prices in recent years. U.S. insurers, especially very large employers, like to think that they're getting the best possible deals in drug pricing, but it's very hard for employers to know how much they're actually paying for a drug.

The net price after all rebates and discounts can only be known weeks or months after the drug bill has been paid. There's very little transparency in this process. The negotiation proposed in H.R. 3 would provide employer with a transparent maximum price. Alternatively, employers could opt out of this price and choose the deal that is best for them.

Patients have no idea why drugs cost so much. For patients, lower and transparent prices would really reduce what they must pay. Patients cost share is typically based on the list price set by the drug company. As a result, most patients do not benefit from these prices that PBMs have negotiated on their employer's behalf when they pay their cost share.

Having a transparent and lower drug price allows all patients to benefit directly from the negotiations. This is especially important for the 30 percent of American workers who are enrolled in a high-deductible health plan. These workers pay the drugs full cost until they meet their deductible.

H.R. 3 allows the secretary to negotiate on behalf of all Americans, and the more people included in the negotiation the greater the negotiating power and the greater the ability to get lower prices. During the COVID–19 pandemic we saw an example of how negotiation can work when the secretary negotiates drug prices.

The secretary negotiated prices for antiviral Remdesivir for example, and then made these prices available to private hospitals who would then directly purchase the drug. These negotiations were possible in a very extreme situation. The drug was urgently needed and there was absolutely no competition available.

The secretary was able to guarantee the purchase of a second quantity of the drug even if private hospitals were the ones really in charge of actually purchasing the drug. This is a very useful model for price negotiations of drugs that don't have competition, but have a great public health interest. Thank you so much for your time and I look forward to answering your questions.

[The prepared statement of Dr. Socal follows:]

PREPARED STATEMENT OF MARIANA SOCAL

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BEFORE THE U.S. CONGRESS

HOUSE COMMITTEE ON EDUCATION AND LABOR

Subcommittee on Health, Employment, Labor and Pensions

Congressional Hearing

" LOWER DRUG COSTS NOW: EXPANDING ACCESS TO AFFORDABLE HEALTH CARE "

Statement of

Mariana P. Socal, M.D., Ph.D.

May 05, 2021

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INTRODUCTION

Chairman DeSaulnier, Ranking Member Allen, and members of the Committee. It is a great honor to be speaking with you today. My name is Dr. Mariana Socal, and I am a medical doctor with a specialization in neurology. I also have a Ph.D. in Health Systems from the Johns Hopkins University and a master's in Public Policy from Princeton University.

I am currently a faculty member in the Department of Health Policy & Management at the Johns Hopkins Bloomberg School of Public Health. My primary research interest is how to improve drug access and affordability for people who need prescription drugs to improve their health and quality of life.

In the last few years, I have received funding to work with several large organizations that are attempting to control drug spending. These include PBGH, the Purchaser Business Group on Health, and ERIC-The ERISA Industry Committee. I have done extensive research examining the drug benefits that self-insured employers – from school districts to America's largest corporations – offer to their workers.

I am speaking today on my own behalf. The opinions expressed herein are my own and do not necessarily reflect the views of The Johns Hopkins University. I would like to provide commentary on how high drug prices impact American employers, their workers, and retirees.

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PART I – HOW HIGH DRUG PRICES IMPACT AMERICAN EMPLOYERS, WORKERS, AND RETIREES

Most Americans obtain health coverage through their employers

Currently, over half of all Americans obtain health coverage through their employer[1] and one third are covered by employer self-sponsored health insurance plans.[2] This means that the prescription drug costs of most American workers are paid by employers and their employees.[3] Both employers and employees are unhappy with the ever-increasing level of spending on drugs. In order to limit their spending, many companies are pushing more and more of the costs of prescription drugs onto the employees. This one of the reasons why members of Congress are hearing more about the cost of prescription drugs from their constituents.

Self-insured employers take a financial risk to cover their employees

Given the high number of Americans who depend on self-insured employers to obtain their coverage, and the financial risk that these employers and employees are taking, it is imperative to keep prescription drug spending under control, not just for the public programs like Medicare and Medicaid, but also for the private sector.

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[1] US Census Bureau - Health Insurance Coverage in the United States: 2018
https://www.census.gov/content/dam/Census/library/publications/2019/demo/p60-267.pdf
[2] Kaiser Family Foundation Employer Health Survey 2018
https://www.kff.org/health-costs/report/2018-employer-health-benefits-survey/
https://www.kff.org/health-costs/report/2018-employer-health-benefits-survey/
[3] Self-insured employers may purchase stoploss insurance, which may cover varying portions of the risk. (reference: Kaiser Family Foundation survey)

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Today, prescription drug prices are on the rise and this means that many Americans are not able to afford the drugs they need, even if they have health insurance because of the out of pocket costs that are associated with many drugs.

Today, most employers negotiate drug prices through a PBM

The typical self-insured employer hires a pharmaceutical benefit manager – PBM – to manage their drug benefit. The PBM negotiates prices with drug manufacturers and, based on these negotiations, the PBM designs the drug formulary that determines the employer's drug benefits. PBMs cannot effectively negotiate lower prices for drugs when there is only one drug to treat a medical condition.

PBMs must have an alternative available in order to successfully negotiate drug prices

In order to obtain a lower price for a certain drug, the PBM offers to place that drug in a favorable position in the formulary – at lower cost sharing or without clinical requirements for utilization. A different drug is given a worse placement on the formulary and both drugs compete for the better placement by offering lower prices. Often, in exchange for a lower price, the PBM may agree to exclude the drug's competitors from the formulary. Therefore, the ability have alternatives and exclude certain drugs from the formulary is *crucial* for the success of most price negotiations performed by PBMs today. When the PBM has a choice, and therefore the ability to negotiate, the market can work. This occurs when there are both branded and generic products available in the market for the same drug, or when there are many

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similarly effective drugs available in the market in the same therapeutic class to treat the same disease.

The market fails when there is no competition

The problem occurs when there is no competition because a certain drug is the only option available in the market. This may occur for new drugs. Of greater concern are drugs that have been on the market for a long period of time. In the United States prices tend to increase after the drug has been launched while in other countries the prices goes down. This is one reason why the prices for many blockbuster drugs are so much higher in the United States than other countries. Drug companies increase the prices in the United States and lower them in other countries.

Drugs can keep increasing their prices by keeping their competitors off the market. This can be accomplished by patent thickets, pay for delay, and other approaches drug companies use to keep generic or even other branded companies from entering the market. There are many different ways to do this but one approach is to add patent terms to the drug by implementing tweaks to the drug's original chemical composition, to the drug's administration mechanism, to the drug's method of use, and so on. Insulin, for example, is an unpatented drug used to treat diabetes. However, the devices used to administer the drug are protected by patents. It is not always the drug that gets the patent protection. Another example is EpiPen.

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Drugs only work if people can afford them

The positive impact from prescription drugs has been widely documented. Drugs can help save lives, prevent diseases, and improve quality of life. However, in order to obtain all these benefits patients must adhere to their treatments and take the drugs that they need in the correct dose and for the appropriate period of time as prescribed. When patients cannot afford their drugs, they cannot adhere to their treatments, and may develop complications. It is estimated that 3 out of 10 US adults did not take their medicines as prescribed in the last year because of the cost. Even more importantly, 1 out of 10 US adults experienced worsening of their condition as a result of not being able to take the drug as recommended.[4]

PART II – HIGH DRUG PRICES DISPROPORTIONATELY AFFECT US WORKERS AS COMPARED TO OTHER COUNTRIES

The US pays much higher prices than other countries for certain drugs

When the market is working the United States pays reasonable prices for drugs. Perhaps the best example is generic drugs, which represent 90% of all drugs sold in the United States. The prices for most generic drugs are comparable to international prices because there is competition. In other cases, there is no competition and the United States pays much higher prices for these drugs.

[4] Kirzinger A, LopesL, Wu B, Brodie M. Kaiser Family Foundation Health Tracking Poll – February 2019: Prescription Drugs. March 01, 2019. https://www.kff.org/health-costs/poll-finding/kff-health-tracking-poll-february-2019-prescription-drugs/

My colleagues and I examined the 79 top-spending drugs in the Medicare Part D program that had no real competition because there were no generics or biosimilars available.[5] These 79 drugs alone were responsible for over half of the Medicare Part D program spending in 2016. We compared the U.S. prices of these drugs to the prices in the UK, in Japan and in Ontario, Canada. We found that, on average, U.S. prices were 3 to 4 times higher than the prices in other countries, for the same drugs.

Other analyses have found very similar estimates. The Ways and Means Committee found that US prices were on average 3.7 times higher than the mean price of 11 other countries.[6] Individual drug prices varied from 70% to 4,833% higher than the mean price in the other 11 countries. More recently, the Government Accountability Office found that US prices for 20 branded drugs were 2 to 4 times higher than in three comparison countries.[7] Drugs with high prices have a number of similarities.

Drugs that have been on the US market for a long time have the highest price differentials when compared to other industrialized countries

While prices in other countries only go down over time, in the US, drug prices tend to go up. The result is that "older" drugs, i.e., those drugs that have been available in

[5] Kang SY, DiStefano MJ, Socal MP, Anderson GF. Using External Reference Pricing in Medicare Part D To Reduce Drug Price Differentials With Other Countries. Health Aff (Millwood). 2019 May;38(5):804-811.

[6] Ways and Means Committee. A Painful Pill to Swallow: U.S. vs. International Prescription Drug Prices. September 2019. https://waysandmeans.house.gov/sites/democrats.waysandmeans.house.gov/files/documents/U.S.%20vs.%20International%20Prescription%20Drug%20Prices_0.pdf

[7] Government Accountability Office. U.S. Prices for Selected Brand Drugs Were Higher on Average than Prices in Australia, Canada, and France. https://www.gao.gov/products/gao-21-282

the US for longer periods of time are the ones that have the highest price differentials when compared to other countries. In our study, for example, each additional year that a drug was in the US market was associated with a 33% higher price differential as compared to the UK, 25% higher price differential as compared to Ontario, Canada, and 17% higher as compared to Japan.[8]

Drugs that have succeeded in continually increasing their prices in the United States, while lowering their prices over time in other countries are a big part of the problem. In January of 2021, a record number of 832 drugs raised their prices in the United States.[9] 99% of these drugs were branded, and most of them had also increased their price for at least the last 2 years.

US drug rebates do not offset the price differential with other countries

Drug companies will argue that they do not get the prices they charge because some of this is taken up by rebates and other price concessions that they must give to get their drug on the formulary. One problem with this argument is that people often have their cost sharing based on the price the drug company sets for the drug and so when the drug company raises their prices the patient pays more.

[8] Kang SY, DiStefano MJ, Socal MP, Anderson GF. Using External Reference Pricing in Medicare Part D To Reduce Drug Price Differentials With Other Countries. Health Aff (Millwood). 2019 May;38(5):804-811.
[9] Marsh T. 800+ Drugs Became More Expensive This January — The Largest Number of Increases in Years. GoodRX Blog. February 02, 2021. https://www.goodrx.com/blog/january-2021-drug-increases-recap/

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Rebates can lower the prices paid by insurers. In our analysis, we accounted for drug rebates paid by drug manufacturers. We found that, in order for the US price to match the average price of the three other countries that we studied, drug manufacturers would have to offer in the US approximately 78% rebate, on average, for the 79 best selling drugs that we studied. This analysis was replicated by the Ways and Means committee and they estimated that the average US rebate would need to be about 73% in order for prices to match the average of the 11 countries that they examined.[10] Drug rebates are confidential, and so it is not possible to verify manufacturer's actual behavior. However, it is unlikely that drug manufacturers would provide such high rebates to the drugs that have been studied because these drugs lacked direct competition. The numbers published by Medicare show average rebates for branded drugs in the low 20%.[11]

Prices before rebates determine Americans' cost-sharing amounts

Even if a manufacturer were to offer a large rebate to the PBM or self-insured company on one of these high-cost drugs, the problem is that the level of cost sharing by the American worker is determined by a drug's pre- rebates prices. The Associated Press reported in the first 7 months of 2018 that drug companies were 96 times more likely to increase the list price than to lower the list price.[12]

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[10] Ways and Means Committee. A Painful Pill to Swallow: U.S. vs. International Prescription Drug Prices. September 2019. https://waysandmeans.house.gov/sites/democrats.waysandmeans.house.gov/files/documents/U.S.%20vs.%20International%20Prescription%20Drug%20Prices_0.pdf

[11] CMS. 2014 Part D Rebate Summary for All Brand Name Drugs. https://www.cms.gov/Research-Statistics-Data-and-Systems/Statistics-Trends-and-Reports/Information-on-Prescription-Drugs/PartD_Rebates.html

[12] https://www.apnews.com/b28338b7c91c4174ad5fad682138520d

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Americans are increasingly required to pay a percentage of the price of their drugs, especially for high-cost specialty drugs.

The amount that patients pay for high cost specialty drugs is frequently calculated as a percentage of a drug's cost. On average, patients pay approximately 22% of the cost of any given drug.[13] This is why it is becoming increasingly hard for Americans to afford drug prices. Patients do not directly benefit from drug rebates because their out-of-pocket payment is typically calculated over the drug's price before rebates. Consider a drug costing $2.1 million.[14] It is for treatment of muscular atrophy, a disease that affects newborns and leaves them unable to walk. Parents are told there is a drug that could help your child walk, but the drug costs $2.1 million and while the insurers will pay 80% of the cost you would still have a $400,000 bill. How many young families can afford $400,000?

Americans pay higher out-of-pocket costs for their drugs than patients in other countries

US insurers are paying higher drug prices than in other countries, and they are increasingly passing on these costs to consumer. As a result, US patients are also paying more out-of-pocket than people in other countries. While in Australia, for example, patients would pay either 5 or 28 dollars for a certain drug, the GAO

[13] IQVIA institute. Estimate of Medicare Part D Costs After Accounting for Manufacturer Rebates. October 2016. https://www.iqvia.com/-/media/iqvia/pdfs/institute-reports/estimate-of-medicare-part-d-costs-after-accounting-for-manufacturer-rebates.pdf

[14] Fidler B. First oral drug for spinal muscular atrophy approved by FDA. Biopharmadive. August 07,2020. https://www.biopharmadive.com/news/roche-ptc-risdiplam-approval-spinal-muscular-atrophy/583167/

estimated that US consumers would pay between 22 and 514 dollars for the same drug in 2018.[15]

Out-of-pocket caps alleviate, but do not necessarily resolve the problem

Fortunately, there are out-of-pocket maximums for most employees with employer-sponsored coverage. However, in about 20% of cases, the out-of-pocket maximum is equal to or higher than $6,000 a year.[16] This amount represents almost 10% of the median household income in America (which, according to the US Census Bureau, was $61,372 in 2017).[17] In addition, patients pay full list price for their drugs while they are on their deductible phase; this is extremely important for the American workers enrolled in high-deductible health plans. As of 2018, 29% of workers with health insurance had high deductible health plans.[18]

Medicare beneficiaries do not have an out-of-pocket maximum

It should also be noted that, while most employees covered by employer-sponsored health insurance are protected by an out-of-pocket maximum, Medicare beneficiaries do not. Medicare beneficiaries obtain their drug benefit through the part D program, which does not have an out of pocket limit. There have been multiple proposals to

[15] Government Accountability Office. U.S. Prices for Selected Brand Drugs Were Higher on Average than Prices in Australia, Canada, and France. https://www.gao.gov/products/gao-21-282
[16] Kaiser Family Foundation Employer Health Survey 2018 https://www.kff.org/health-costs/report/2018-employer-health-benefits-survey/
[17] US Census Bureau https://www.census.gov/library/publications/2018/demo/p60-263.html
[18] Kaiser Family Foundation Employer Health Survey 2018 https://www.kff.org/health-costs/report/2018-employer-health-benefits-survey/

limit the out-of-pocket liability for Medicare beneficiaries; these proposals simply disagree on the amount of the out of pocket maximum.

PART III – H.R.3 AND OTHER POLICY CONSIDERATIONS

High drug prices strain American employers, workers, and retirees. The market does not work for certain drugs because the PBMs, the main negotiators in the US system, have limited negotiating power when there is no competition. For these cases, alternative negotiation pathways are greatly needed. The negotiation mechanisms outlined in H.R.3 target these drugs for which there is a market failure. In the absence of product-to-product competition within the US market, the price comparison between the US and other countries can offer an alternative pathway for negotiation.

Using international prices as a benchmark can bring the US price back to international norms

Currently, most pharmaceutical manufacturers are global companies and they rely on sales in both US and international markets to obtain their revenue.[19] Using average international market prices as a benchmark for US price negotiations will generate significant savings for US employers and their employees. Our analysis of the 79 top-spending drugs in Medicare part D found that, if the US paid the average price

[19] https://www.whitehouse.gov/wp-content/uploads/2017/11/CEA-Rx-White-Paper-Final2.pdf

across the countries that we studied, savings would be $72.9 billion dollars in 2018.[20] The Ways and Means Committee estimated $49 billion in savings per year for Medicare Part D alone.[21] If employers adopted this approach the savings would be similar.

Other countries are unlikely to raises the prices that they pay for drugs; in fact, the prices keep getting lower. In the US, drug prices keep getting higher. There is no reason why the US should be paying 3-4 times what other countries are paying for the same drug.

Which countries should be included in the international price?

It is important to select countries that have similar per capita incomes and large pharmaceutical markets like the US. They can afford the expansive drugs. Ideally, these countries would also have diverse price-setting approaches. For example, some countries such as the UK have value-based pricing, whereas other countries such as Germany have market-based pricing. Our research found no major differences in the prices that are determined by the different approaches. Although countries may have different mechanisms for setting or negotiating drug prices, ultimately they obtain drug prices within the same price range.

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[20] Kang SY, DiStefano MJ, Socal MP, Anderson GF. Using External Reference Pricing in Medicare Part D to Reduce Drug Price Differentials With Other Countries. Health Aff (Millwood). 2019 May;38(5):804-811.

[21] Ways and Means Committee. A Painful Pill to Swallow: U.S. vs. International Prescription Drug Prices. September 2019. https://waysandmeans.house.gov/sites/democrats.waysandmeans.house.gov/files/documents/U.S.%20vs.%20International%20Prescription%20Drug%20Prices_0.pdf

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There is strength in numbers: price negotiations involving more individuals result in lower drug prices

Currently, negotiations for most covered Americans are fragmented. HR 3's proposal of having the HHS Secretary negotiate on behalf of all Medicare beneficiaries and those covered by private insurers, including by self-insured employers, would greatly increase the negotiation power because it would cover the vast majority of Americans. Combining larger numbers of individuals in a single negotiation has been shown to increase negotiating power and result in lower drug prices.[22] In addition, companies can opt out of the negotiated price if they can get a better deal, which is a critical element of this proposal.

Experience suggests that the HHS Secretary can successfully negotiate prices

The experience of governmental agencies such as the Department of Veterans Affairs and the Department of Defense provides a solid example in support of the HHS Secretary successfully negotiating drug prices. These agencies have negotiated drug prices on behalf of their beneficiaries for years and have obtained the lowest prices in America today.[23] It is estimated, for example, that the VA pays 44% less than Medicare for a same basket of drugs,[24] and, the VA purchases a lot fewer drugs than Medicare.

[22] Insurer bargaining and negotiated drug prices in Medicare Part D. Lakdawalla D., Yin W. NBER Working Paper 15330. http://www.nber.org/papers/w15330
[23] GAO-13-358. Prescription Drugs:Comparison of DOD and VA Direct Purchase Prices. https://www.gao.gov/products/GAO-13-358
[24] Venker B, Stephenson KB, Gellad WF. Assessment of Spending in Medicare Part D If Medication Prices from the Department of Veterans Affairs Were Used. JAMA Intern Med. 2019;179(3):431-433.

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There is strong public support for allowing the HHS Secretary to negotiate drug prices

The Kaiser Family Foundation performs a periodic survey of the American public to examine the public's opinions, knowledge, and experiences on various issues related to the U.S. health care system.[25] In February 2019, the Kaiser Family Foundation survey found that 86% of the general public and 82% of Americans aged 65 and older supported allowing the federal government to negotiate with drug companies to get a lower price for people on Medicare. My own research has shown that 60% of older Americans would even trade off the possibility of choosing or changing a drug plan in Medicare Part D for more affordable drug prices.[26]

Having the HHS Secretary negotiate drug prices would benefit employers

Currently, many Medicare prescription drug plans are managed by the same PBMs who manage the drug benefit for private plans, including for self-insured employers.[27] This means that when PBMs can't negotiate effectively for Medicare plans, they can't negotiate effectively for private plans, and vice versa.

Employers need help getting good prices for high-cost drugs

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[25] KFF Health Tracking Poll – February 2019: Prescription Drugs. https://www.kff.org/health-costs/poll-finding/kff-health-tracking-poll-february-2019-prescription-drugs/

[26] Socal MP, Anderson GF.
Older Americans' Preferences Between Lower Drug Prices and Prescription Drug Plan Choice, 2019. Am J Public Health. 2020 Mar;110(3):354-356.

[27] Insurer bargaining and negotiated drug prices in Medicare Part D. Lakdawalla D., Yin W. NBER Working Paper 15330. http://www.nber.org/papers/w15330

US companies, especially very large employers, like to think that they are getting the best possible deals from their PBMs. However, this is not always the case. We were asked by ERIC, the Committee that represents large nationwide employers who are also plan sponsors, to examine the prices that 10 of the largest US corporations were paying for biologics and biosimilars. The first thing that we found was that the PBMs did not always give these companies the information they needed to determine if they were getting a good deal. When we finally got the data, we found that two companies of the same size and using the same PBM were paying about 10% different prices for a same high-cost biologic drug.[28]

Employers and workers are spending unnecessarily high amounts on branded drugs. Increased price transparency can help reduce that differential, but not eliminate it

PBMs have a financial incentive to keep high-cost, high-rebate drugs in their employers' drug formularies. This is because, for branded drugs, PBMs can make a profit by retaining some portion of the rebates plus any fees that they obtain from drug manufacturers, and drugs that are more highly priced can generally offer greater rebates. Therefore, drugs that have high prices and high rebates may be favored in the formulary in detriment of lower-cost alternatives. In the Medicare program, for example, we found that 70% of part D prescription drug plans had placed at least one branded drug placed more favorably in the formulary than its corresponding generic.[29] This increases cost unnecessarily for both plans and beneficiaries.

[28] These are initial results from an ongoing research project and have not been published.

[29] Socal MP, Bai G, Anderson GF. Favorable Formulary Placement of Branded Drugs in Medicare Prescription Drug Plans When Generics Are Available. JAMA Intern Med. 2019 Jun 1;179(6):832-833.

Unfortunately, employers do not always have the full information to identify that these distortions are present in their drug formulary. [30]

Reducing wasteful spending from high-price high-rebate drugs could save employers up to 24% of their overall pharmacy spending

An analysis of 15 large US companies by the Pacific Business Group on Health, a purchaser coalition representing 60 public and private organizations across the U.S that collectively purchase healthcare for 10 million Americans,[31] has shown that reducing the use of high-cost, low-value drugs could save 3% to 24% of a company's *overall* pharmacy spending. [32] Having a transparent price for branded drugs available for all employers would increase transparency and would help employers identify where they are spending too much with certain drugs, better equipping employers to identify and ultimately remove wasteful spending from their drug benefit.

Having the option of accessing the HHS-negotiated price would benefit employers in two ways: lower drug prices and increased transparency

Having the option of accessing the federally-negotiated price would, first, offer lower prices to employers and to workers who obtain coverage through employer-sponsored health insurance. PBMs would still be allowed to negotiate down prices,

[30] Bai G, Socal MP & Anderson GF. Policy Options To Help Self-Insured Employers Improve PBM Contracting Efficiency. Health Affairs Blog. May 29, 2019. https://www.healthaffairs.org/do/10.1377/hblog20190529.43197/full/
[31] http://www.pbgh.org/about/members
[32] Vela, L. Reducing Wasteful Spending in Employers' Pharmacy Benefit Plans. https://www.commonwealthfund.org/publications/issue-briefs/2019/aug/reducing-wasteful-spending-employers-pharmacy-benefit-plans

bringing additional price reductions into the system. The experience in the Japanese system, where the government negotiates a maximum price and payers obtain further discounts from their own subsequent negotiations, shows that drugs' actual selling prices will be lower than the maximum price in the government fee schedule because of competition among distributors.[33] In addition, HR 3 would benefit employers by providing them with a transparent maximum price. Having a transparent pricing benchmark will show employers if they are getting a better deal by opting in or opting out, improving their decision-making.

There is recent evidence that the government can negotiate prices for private purchasers

During the COVID-19 pandemic, the HHS Secretary negotiated prices for drugs and vaccines. The experience of the drug Remdesivir, an antiviral used at the hospital setting to treat COVID-19, provides a helpful example of how government-negotiated prices can be made available to private purchasers. In the case of remdesivir, the federal government allocated purchasing quotas to states and states were in charge of allocating these quotas to hospitals. Hospitals were in charge of purchasing the drug, when they could take advantage of the government-negotiated price, and were reimbursed by insurers accordingly.[34] Purchasing quotas were needed in this case because the supply of remdesivir was very limited. For branded drugs – especially those that have been in the US market for many years – it is unlikely that purchasing quotas would be needed.

[33] Ikegani N, Anderson GF. In Japan, All-Payer Rate Setting Under Tight Government Control Has Proved To Be An Effective Approach To Containing Costs. Health Aff (Millwood). . 2012 May;31(5):1049-56.
[34] Socal MP, Anderson GF. The Role of Advance Purchasing Commitments in Government Drug Price Negotiations: Lessons From the COVID-19 Response. Am J Public Health. 2021 Apr;111(4):652-657.

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For patients, greater price transparency may reduce cost-sharing

Currently, when beneficiaries must pay a percentage of the drug cost, the patient's cost-sharing amount is calculated based on the drug's list price (i.e., the price before rebates and discounts are applied). The drug's net price after rebates and discounts is usually not known at the time that the patient is obtaining their drug and therefore it cannot be used. HR 3 will allow for HHS-negotiated prices to be available at the time that patients are obtaining their drug, allowing these prices to be used in cost sharing calculations. HHS-negotiated prices are likely to be much lower than the list price, which would likely translate to lower cost-sharing amounts for patients.

Having a penalty is an important element to enable the negotiation

The US pays more than other countries especially for drugs that have been on the market for many years. When drugs already have an established market, and there are patients who depend on them, PBMs are less likely to be able to say "no" and remove the drug from the formulary. Therefore, some drugs may exhibit egregious price-hiking behaviors such as Martin Skhreli's Daraprim's overnight 5000% price increase back in 2015 without concerns for losing market share.[35] It is important to have a clear penalty that can prevent these behaviors and ensure that drug manufacturers come to the table to negotiate.

[35] https://khn.org/news/for-shame-pharma-bro-shkreli-is-in-prison-but-daraprims-price-is-still-high/

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Having an inflationary rebate is an important mechanism to prevent price hikes for drugs that are not eligible or not selected for negotiation

Not all drugs will be selected for negotiation in a given time period. However, they may still exhibit price increases. H.R.3 establishes an inflationary rebate that provides an important mechanism to prevent such price increases for branded and generic drugs alike.

In order to protect and reward innovation, new drugs are granted patents that provide a period of time in which the drug has a monopoly i.e., no other competitor may enter the market. Drug manufacturers set the drug's launch price to allow them to recoup their research and development investments during the drug's monopoly period. Price changes that occur after a drug has launched are unlikely to be related to the need to recoup R&D investment. Other developed countries have mechanisms in place to prevent this type of behavior. In the US, many of today's high-cost drugs originally entered the market at lower prices and have only become expensive over time.

Negotiating price and quantity would offer an incentive for manufacturers to negotiate

The price negotiations established in H.R.3 represent an important tool to bring down drug prices, especially for drugs that have been in the US market for several years and have large price differentials with other countries. However, such price negotiation mechanisms are ineffective for drugs that lack any form of competition

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or are not yet available in other countries. In such cases, the current PBM-based negotiation model and the H.R. 3 international-price proposed model might not necessarily provide effective tools for negotiation. An alternative negotiation tool could be to negotiate price and quantity simultaneously, such as through advance purchasing commitments.[36]

Simultaneously negotiating both the price and the quantity of a drug may provide an incentive for drug manufacturers to participate in the negotiations and offer price concessions in exchange for increased revenue certainty. This is how retailers like Walmart are able to negotiate lower prices, and states have also sought such mechanisms when purchasing drugs. In addition, purchasing agreements established by the HHS Secretary with drug manufacturers during the Covid-19 pandemic provide examples of how such negotiations can be successfully implemented. The negotiation for the antiviral drug remdesivir achieved US price to government purchasers at the same level of prices offered to other countries, and prices to US private purchasers 33% higher than prices offered to other countries – a price close to the 120% established as the negotiation threshold by H.R. 3.[37]

This experience also shows that the government does not have to actually purchase the drug but can simply guarantee a certain volume of sales. If the committed quantity were not to be realized over the defined time period, the federal

[36] The Role of Advance Purchasing Commitments in Government Drug Price Negotiations: Lessons From the COVID-19 Response. Socal MP, Anderson GF. Am J Public Health. 2021 Apr;111(4):652-657.
[37] The Role of Advance Purchasing Commitments in Government Drug Price Negotiations: Lessons From the COVID-19 Response. Socal MP, Anderson GF. Am J Public Health. 2021 Apr;111(4):652-657.

government could pay for the remaining negotiated quantity and utilize the leftover amount to provide care for specific programs or populations – such as uninsured patients or the prison system – or to stockpile it for future use. Finally, it allows everyone to have access to the drug at the negotiated price.

PART IV – IMPACT ON INNOVATION AND FOREIGN PRICES

Drug costs are unlikely to shift to other countries if the Secretary uses an international benchmark to negotiate prices in the US

Most developed countries have mechanisms in place to negotiate or regulate drug prices.[38] For example, the UK has a system of value-based pricing based on health technology assessment. In this system, a drug's benefits are compared to the other drugs that are available in the market for the same condition. The drug's price is then determined according to the value that the drug adds in comparison to its therapeutic alternatives. Such mechanisms are unlikely to be influenced by the US decision to include the country's price in the international benchmark. In addition, most countries already reference other countries' drug prices when negotiating or setting drug prices domestically.[39] Comparing prices to what other countries pay is not a new idea.

[38] Maniadakis N, Kourlaba G, Shen J, Holtorf A. Comprehensive taxonomy and worldwide trends in pharmaceutical policies in relation to country income status. BMC Health Serv Res. 2017; 17: 371.
[39] OECD Health Policy Studies. Pharmaceutical Pricing Policies in a Global Market. September 24, 2008. https://www.oecd.org/els/pharmaceutical-pricing-policies-in-a-global-market.htm

A potential unintended consequence of this practice, however, is that drug manufacturers could choose to delay[40] or not launch in a certain product in a given country - if they know the country will be used as a reference - in order to maintain the average price high. This is mostly a concern when including countries with less developed pharmaceutical markets in the international price. If only major pharmaceutical markets are included in the international price, manufacturers are highly unlikely to choose not to launch their product in that country.[41] It is hard to keep Germany, Japan, France and the United Kingdom out of the market and only sell in the US.

Allowing the HHS Secretary to negotiate drug prices is unlikely to significantly discourage drug innovation

The concern that negotiating prices would discourage innovation comes from the perception that, if pharmaceutical manufacturers were to have lower revenue, they would have insufficient funds or lack the incentives to invest in research and development of new drugs. In their analysis of H.R. 3, the Congressional Budget Office has estimated that about 8 fewer drugs would be launched in the US and the global market.[42] However, it is unknown how truly innovative these drugs would be. The impact of price negotiations may be greater on less innovative or useful drugs. This may actually steer drug research and development into innovative areas. There

[40] Congressional Budget Office. Budgetary Effects of H.R. 3, the Elijah E. Cummings Lower Drug Costs Now Act. December 10, 2019. https://www.cbo.gov/system/files/2019-12/hr3_complete.pdf
[41] OECD Health Policy Studies. Pharmaceutical Pricing Policies in a Global Market. September 24, 2008. https://www.oecd.org/els/pharmaceutical-pricing-policies-in-a-global-market.htm

[42] Congressional Budget Office. Budgetary Effects of H.R. 3, the Elijah E. Cummings Lower Drug Costs Now Act. December 10, 2019. https://www.cbo.gov/system/files/2019-12/hr3_complete.pdf

is no reliable way to know what will happen, but we know that innovation is key to the branded companies and they will continue to innovate.

Without innovation, drug manufacturers have nothing to sell

Having a strong drug development pipeline is crucial in order to attract investors and remain competitive in the market. Companies like Pfizer, Merck, and J&J need to continually bring new products. A company that relies on US profits from its existing drug portfolio will be left behind by bold start-ups and foreign competitors if the company stops innovating. Claiming that controlling unreasonably high drug prices will hamper innovation flies in the face of how basic business and scientific incentives work.

Drug research and development is not the main reason for high drug prices

It is generally assumed that manufacturers set drug prices at levels that allow them to recoup drug research and development costs, not only of the drug in question but also for all other drugs that failed in the pipeline. However, there is growing evidence that research and development costs are not the main drivers of high drug prices.

In recent investigations, the House Committee on Oversight and Reform examined price-setting behaviors of different pharmaceutical manufacturers. These investigations have found that drug manufacturers often increased drug prices as a

response to low quarterly earnings and to increase executive compensation.[43] The expense of research and development has already occurred when the drug company raises its prices.

Under current prices, drug companies are able to recoup their investments in drug research and development multiple times over

Estimates suggest that, after four years in the market, most drugs will have generated over 9 times higher revenue than their own research and development costs.[44]

High drug prices do not necessarily mean greater clinical value

When evaluated for their safety and effectiveness in comparison to the other drugs available in the market, the vast majority - approximately 75% - of the specialty drugs sold in the US does not provide added therapeutic value as compared to conventional therapy. These are among the most expensive drugs in the US, and represent a large portion – about 15% – of Medicare part D spending[45]

Public funds support a large proportion of drug innovation

[43] House Committee on Oversight and Reform. Committee Releases Additional Staff Reports on Skyrocketing Drug Prices for Day 2 of Landmark Hearings with CEOs. Ocober 01. 2020. https://oversight.house.gov/news/press-releases/committee-releases-additional-staff-reports-on-skyrocketing-drug-prices-for-day

[44] Prasad V., Mailankodi S. Research and Development Spending to Bring a Single Cancer Drug to Market and Revenues After Approval. JAMA Intern Med. 2017;177(11):1569-1575. ttps://jamanetwork.com/journals/jamainternalmedicine/fullarticle/2653012

[45] DiStefano, M. J., Kang, S. Y., Yehia, F., Morales, C., & Anderson, G. F. (2021). Assessing the Added Therapeutic Benefit of Ultra-Expensive Drugs. Value in Health, 24(3), 397-403.

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A large scientific literature shows that most new drugs originate as scientific breakthroughs from research funded by the federal government, though its agencies such as the National Institutes of Health (NIH). An analysis found that 97% of all new drugs approved by the FDA from 2010 to 2016 had received NIH support for the identification of the drug or its mechanistic basis.[46] The same study also found that 93% of the 100 most commonly prescribed drugs in the US had received NIH support. Government funding is especially critical at the initial phases of drug development, when failure rates are high. If the savings obtained from price negotiations were reinvested, governmental funding for drug discovery and development could be expanded.

Drug manufacturers spend more on advertisement than on drug development

Drug research and development costs represent a small portion of drug manufacturers' total spending. Pharmaceutical manufacturers spend more on drug marketing than they do on drug research and development. Nine out of 10 big pharmaceutical companies spend more on marketing than on research.[47] Even if manufacturer revenues were to decrease under the new policy, manufacturers would be unlikely to choose to cut spending on drug development when they could first implement cuts to the marketing budgets.

[46] Griesenauer RH, Moore R, Kinch MS. NIH Support for FDA-Approved Medicines. Cell Chemical Biology Volume 24, ISSUE 11, P1315-1316, November 16, 2017. https://www.cell.com/cell-chemical-biology/fulltext/S2451-9456(17)30397-5

[47] Swanson A. Big pharmaceutical companies are spending far more on marketing than research. https://www.washingtonpost.com/news/wonk/wp/2015/02/11/big-pharmaceutical-companies-are-spending-far-more-on-marketing-than-research/

FINAL REMARKS

High drug prices strain American employers, workers, and retirees. Because most Americans obtain health insurance through their employers, lowering US health care costs not only helps bring down premiums and out-of-pocket payments; lower health care costs also contribute to making American workers and corporations more competitive in the global market.

Thank you so much. I look forward to answering any questions that you may have.

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Chairman DESAULNIER. Thank you, Doctor. We'll now go to Mr. Mitchell. Mr. Mitchell the floor is yours.

STATEMENT OF DAVID MITCHELL, FOUNDER AND PRESIDENT, PATIENTS FOR AFFORDABLE DRUGS

Mr. MITCHELL. Chairman DeSaulnier, Ranking Member Allen, Members of the Committee. I'm David Mitchell. I'm founder of Patients for Affordable Drugs. More importantly, like you Mr. Chairman, I have an incurable blood cancer, and prescription drugs are keeping me alive.

My doctors have me on a four-drug combination right now with a list price of more than $900,000 per year. Just one of my oral drugs called POMALYST is priced at more than $20,000 for 21 capsules. And because Medicare beneficiaries like me pay our costs based on list price, I spent more than $18,000 out of pocket last year just for POMALYST.

For people with my cancer, multiple myeloma, drugs account for 60 percent of the cost of treatment. I'm a very lucky man. These drugs are keeping my cancer at bay, but eventually this combination is going to stop working, and I'll need a new treatment.

So I care deeply about innovation and new drug development. But drugs don't work if people can't afford them. Drugs are too expensive in the U.S. with no justification. When drug makers hike prices each year they don't do it because the drug is more valuable, drug companies raise prices because they can. As a result, Americans pay almost four times what other wealthy nations pay for the exact same drugs.

Nearly 40 percent of Americans say it's difficult to afford their medications. 90 percent of voters across both parties say it's extremely important that Congress take action on drug pricing. Now of course biopharma opposes any reforms that would curb its unilateral power to dictate prices.

So it threatens that reforms will destroy innovation and access to new drugs. But these claims don't hold up. Here are four reasons why. One: Biopharma enjoys profits that are almost three times the average of the S&P 500. Brand name drug companies could

lose a trillion dollars in sales over 10 years and remain the most profitable industry in the U.S.

Two: A huge amount of R&D is coming from taxpayers. NIH funded research is associated with every new drug approved by the FDA from 2010 to 2019. COVID vaccines illustrate this point. The unprecedented speed of vaccine development was enabled by more than $17 billion of NIH funded research on vaccine technologies prior to the pandemic.

A new method of developing vaccines was waiting to be tested, then the government invested another $18 billion for clinical trials to stand up production, spending whatever was necessary and eliminating financial risks.

Three: CBO says we can prep pharma revenue by up to a trillion dollars over 10 years and lose only 8 of 300 new drugs. And many of those 8 wouldn't be losses because only 10 to 15 percent of new drugs represent true therapeutic advances.

Four: Big pharma threatens that patients will lose early access to drugs. But drug companies file for approval first in the U.S. because we have the highest prices and the largest market in the world. Given that U.S. prices for brand drugs are almost four times what other wealthy nations pay, we can lower prices and still offer the highest prices by far in the largest market in the world.

Congress must act. H.R. 3 is the comprehensive reform we need. It will lower prices, reign in price gouging and reduce out of pocket costs. Now it's estimated to save the Federal Government over $450 billion. Big pharma complains that redeploying these savings to address other critical needs is tantamount to using the industry as a piggy bank, but in reality it's pharma that has treated patients and taxpayers as piggy banks for years, raising prices at will to hit profit targets and trigger executive bonuses.

Pharma is right about one thing: America does have other priorities and every dollar we send to pharma in unjustified profits is a dollar we don't have to reduce health care disparities, provide health care to the uninsured, or increase NIH research on drugs for rare diseases, like mine.

H.R. 3 enjoys 93 percent bipartisan support. It's time to pass it. Of course it's an uphill fight against powerful lobby let's be clear. Big pharma is not fighting for the interests of patients. Recently, the head of PhRMA, the trade association, affirmed that fact.

He said his industry is 'adept at rolling the tanks to push back against policy proposals adverse to the industry's interest.' So you must choose a side, stand with patients and taxpayers, or stand with pharma to protect the industry's interests. But let's be honest that's what this fight is about. It's about restoring balance to ensure we get the innovation we need at prices we can afford.

You hold the power to make the changes the American people are demanding. We're going to do our part to make sure their voices are heard. Thank you.

[The prepared statement of Mr. Mitchell follows:]

PREPARED STATEMENT OF DAVID MITCHELL

PATIENTS FOR AFFORDABLE DRUGS™

Statement of David E. Mitchell
Founder, Patients For Affordable Drugs

before the

U.S. House of Representatives Subcommittee on Health, Employment, Labor, and Pensions of the

House Committee on Education and Labor
for a hearing on

Lower Drug Costs Now: Expanding Access to Affordable Health Care

May 5, 2021

Chairman DeSaulnier, Ranking Member Allen, Members of the Committee. Thank you for having me today.

Section I. Background and Introduction

My name is David Mitchell. I am the founder of Patients For Affordable Drugs. We are a bipartisan organization focused on policies to lower prescription drug prices. We don't accept funding from any organizations that profit from the development or distribution of prescription drugs.

In just over four years since we launched, we have collected over 27,000 stories of patients struggling to pay high drug prices. And we have built a community of more than 340,000 patients and allies who support policies to lower drug prices.

More importantly for today, I have an incurable blood cancer, and prescription drugs are keeping me alive — literally.

My doctors currently have me on a four-drug combination of infused and oral cancer drugs. These four drugs carry a combined list price of more than $900,000 per year. Just one of my oral

drugs, called Pomalyst, is priced at more than $20,000 for 21 capsules, which I must buy every 28 days. And because Medicare beneficiaries like me pay our costs in Part D based on list price, I spent more than $18,000 out of pocket last year — just for Pomalyst. To help manage the cost of my infused drugs, I spend another $3,000 per year to purchase a Part B supplement. And of course, I have the base costs of Medicare to pay as well. For people with my cancer — multiple myeloma — drugs account for 60 percent of the cost of treatment.[1] Sixty percent.

I am a very lucky man — these drugs are currently keeping my cancer at bay, and I tolerate them well. But the reason I am on four drugs is because each began to stop working, so the doctors first increased the dose, then increased the frequency, and then added another drug. Eventually I will fail on this combination, too. When that happens, I will be what doctors call "triple refractory" to all of the three major classes of drugs used to treat my disease. The cancer will begin to increase in my blood and I will need a new treatment. Fortunately, there are options out there.

But one of the new drugs approved this year that I might be a candidate for carries a list price of $419,500. That's just for the drug — it doesn't cover the hundreds of thousands of dollars required to administer the drug and manage my health in the wake of the treatment.

The point is: I need these innovative drugs. I care deeply about innovation and new drug development. My life depends on it. Without innovation, I will die sooner than I hope to. That is just an unfortunate fact.

But my 10-year journey as a cancer patient has taught me one irrefutable fact: Drugs don't work if people can't afford them.

Section II. The Price of Drugs and Need for Change

Drugs are too expensive in the United States, and there is no justification for the high prices. When drug makers hike prices each year, they don't do so because the drug becomes more valuable. Drug companies raise prices because they can. We let them.

The result is that Americans pay nearly four times what people in other wealthy nations pay for the exact same brand-name drugs.[2]

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[1] Tran, D., Kamalakar, R., Manthena, S., & Karve, S. (2019, November 13). Economic Burden of Multiple Myeloma: Results from a Large Employer-Sponsored Real-World Administrative Claims Database, 2012 to 2018. *Blood*, 134, 3414. https://doi.org/10.1182/blood-2019-131264

[2] House Committee on Ways and Means. (2019). A Painful Pill to Swallow: U.S. vs. International Prescription Drug Prices.https://waysandmeans.house.gov/sites/democrats.waysandmeans.house.gov/files/documents/U.S.%20vs.%20International%20Prescription%20Drug%20Prices_0.pdf

Consequently, nearly 40 percent of people report having difficulty affording their medications.[3] When their prescription drug prices are too high, Americans face challenges affording other expenses, such as food and housing. One survey found that over 20 percent of people took on debt or declared bankruptcy because of their medications.[3]

The issue of drug prices disproportionately harms communities of color. One in two Latinos in the United States take a prescription medication, and 20 percent are uninsured.[4] Black Americans are more likely to live with chronic pain, diabetes, and high blood pressure than white Americans and are nearly two times more likely to be uninsured.[5]

The pandemic only made it worse, as millions of Americans lost jobs, income, and insurance coverage. As expensive as my drugs are even with Medicare, I never lose sight of the fact that 30 million Americans without insurance are exposed to the full list price.[6]

People struggle to pay the prices with and without insurance. Lynn Scarfuto from Herkimer, New York spent 25 years working as a nurse. During nine of those years, she worked with cancer patients helping them access the best treatment possible. After she retired, she was diagnosed with chronic lymphocytic leukemia and was prescribed the cancer medication Imbruvica. It carries a monthly price tag of almost $15,000.[7] She relies on hard-to-obtain, short-term funding for her medication this year. But she doesn't know how she will afford it when her grant money runs out. She lives in fear of how she'll ever afford the astronomical price tag of the medication keeping her alive.[8]

Americans are desperate for relief. A Politico-Harvard poll from early this year found that nearly 90 percent of voters across both parties thought it was "extremely important" that Congress and the president take action on drug pricing. That includes 91 percent of Democrats and over 80

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[3] Nguyen, A. (2021, March 22). *Survey: Americans Struggle to Afford Medications as COVID-19 Hits Savings and Insurance Coverage*. GoodRx. https://www.goodrx.com/blog/survey-covid-19-effects-on-medication-affordability/
[4] UnidosUS Action Fund. (2021). *A Vicious Cycle of Health Inequity: How High Prescription Prices Hurt Latino Health and Prosperity*. https://www.lowerdrugpricesnow.org/wp-content/uploads/UNIDOS-RX-REPORT-Vicious-Cycle.pdf
[5] Patients For Affordable Drugs Now. (2020, December 14). *High Prescription Drug Prices Perpetuate Systemic Racism. We Can Change It*. https://patientsforaffordabledrugsnow.org/2020/12/14/drug-pricing-systemic-racism/
[6] Garfield, R. & Tolbert, J. (2020, September 17). *What We Do and Don't Know About Recent Trends in Health Insurance Coverage in the US*. Kaiser Family Foundation. https://www.kff.org/policy-watch/what-we-do-and-dont-know-about-recent-trends-in-health-insurance-coverage-in-the-us/
[7] Wholesale acquisition cost from AnalySource® as reprinted with permission by First DataBank Inc. All rights reserved. © (2021). Please refer to http://www.fdbhealth.com/policies/drug-pricing-policy/ for more information.
[8] Patients For Affordable Drugs. (2021, March 18). *I don't know how I'll be able to afford my treatment*. https://patientsforaffordabledrugs.org/2021/03/18/lynn-scarfuto/

percent of Republicans.[9] And voters are worried you won't do enough to help them — over 60 percent fear Congress wouldn't go far enough to reform our broken drug pricing system.[10]

You can change all this. You can restore balance to ensure we get the innovation we need at prices we can afford. And you can do it now.

Section III. Innovation and Drug Prices: The False Choice

Of course, the biopharmaceutical industry opposes any reforms that would curb its unilateral power to dictate prices for brand drugs. So it rolls out its well-worn claim that any limits on its ability to set high prices will destroy innovation and access to new drugs.

No one cares more about innovation than patients. But if you pull back the curtain on this fear-mongering, the argument doesn't hold up.

Experts from both sides of the aisle agree it's possible to curb the pharmaceutical industry's pricing power without threatening valuable innovation.[11-12] There are five reasons why:

1) Biopharma corporations enjoy profit margins that are almost three times the average of the S&P 500.[13] Brand-name pharmaceutical companies could lose $1 trillion in sales over 10 years and remain the most profitable industry in the United States.[14] There is more than enough headroom to lower drug prices and leave drug companies with plenty of profit to attract investment and fund research and development. And if drug pricing

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[9] POLITICO & Harvard T.H. Chan School of Public Health. (2021, January). *The American Public's Priorities for the New President and Congress*. https://cdn1.sph.harvard.edu/wp-content/uploads/sites/94/2021/01/Politico-HSPH-Jan-2021-PollReport.pdf

[10] Hart Research Associates. (2021, April 20). Memo for Protect Our Care. https://www.protectourcare.org/wp-content/uploads/2021/04/Garin-Memo-Protect-Our-Care-April-2021.pdf

[11] Frank, R. G. (2019, November 13). Drug companies exaggerate — controlling drug prices won't threaten innovation. *The Hill*. https://thehill.com/opinion/healthcare/470266-drug-companies-exaggerate-controlling-drug-prices-wont-threaten-innovation

[12] Waikar, S. (2020, September 2). *Pharma Companies Argue That Lower Drug Prices Would Mean Fewer Breakthrough Drugs. Is That True?*. Kellogg School of Management at Northwestern University. https://insight.kellogg.northwestern.edu/article/pharma-companies-argue-lower-drug-prices-fewer-breakthrough-drugs

[13] Yardeni Research. (2021, January 19). *S&P 500 Sectors & Industries Profit Margins (quarterly)*. https://www.yardeni.com/pub/sp500margin.pdf

[14] West Health. (2019, November 14). *New Analysis Finds Large Drugmakers Could Lose $1 Trillion in Sales and Still Be the Most Profitable Industry*. https://www.westhealth.org/press-release/new-analysis-finds-large-drug-makers-could-lose-1-trillion-in-sales-and-still-be-the-most-profitable-industry/

legislation curbs profits, the industry can maintain or even increase R&D investment by shifting the billions spent on marketing, advertising, and lobbying.

2) It doesn't cost nearly as much as the industry says it does to develop a new drug. Pharma claims it costs $2.87 billion to bring a new drug to market. But that's based on industry-funded research and undisclosed source data.[15-16] Independent studies have found the cost to develop a drug is likely less than $1 billion.[17-18]

3) A tremendous amount of research and development is coming from taxpayers. The National Institutes of Health (NIH) is the single largest biomedical research agency in the world. NIH-funded research is associated with all 356 new drugs that were approved by the FDA from 2010 to 2019.[19] NIH Director Francis Collins has said: "Finding new treatments thus requires NIH to play a lead role — by investing in the early stage of therapeutic development to 'de-risk' such projects."[20] Drug companies argue high drug prices are required to reimburse the industry for the financial and scientific risk it takes on during research and development. In reality, the U.S. government takes on most of those early risks, further undermining the industry's argument for high prices.

   Our experience with COVID-19 vaccines illuminates this point with crystal clarity.

   Several years back when the big drug companies were unwilling to invest their own money in technology that is leading to some of the most promising vaccines today, the

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[15] DiMasi, J. A., Grabowski, H. G., & Hansen, R. W. (2016). Innovation in the pharmaceutical industry: New estimates of R&D costs. *Journal of Health Economics*, *47*, 20-33. https://doi.org/10.1016/j.jhealeco.2016.01.012
[16] Tufts Center for the Study of Drug Development. (n.d.). *Financial Disclosure*. https://csdd.tufts.edu/financial-disclosure
[17] Wouters, O. J., McKee, M., & Lutyen, J. (2020). Estimated Research and Development Investment Needed to Bring a New Medicine to Market, 2009-2018. *JAMA*, *323*(9), 844-853. https://doi.org/10.1001/jama.2020.1166
[18] Prasad, V., & Mailankody, S. (2017). Research and Development Spending to Bring a Single Cancer Drug to Market and Revenues After Approval. *JAMA Internal Medicine*, *177*(11), 1569-1575. https://doi.org/10.1001/jamainternmed.2017.3601
[19] Ledley, F., Cleary, E., & Jackson, M. (2020, September 2). *US Tax Dollars Funded Every New Pharmaceutical in the Last Decade*. Institute for New Economic Thinking. https://www.ineteconomics.org/perspectives/blog/us-tax-dollars-funded-every-new-pharmaceutical-in-the-last-decade
[20] Collins, F. S. (2017, May 17). *Testimony on the Transformative Power of Biomedical Research*. National Institutes of Health. https://www.nih.gov/about-nih/who-we-are/nih-director/testimony-transformative-power-biomedical-research

U.S. government did.[21-22] The biopharmaceutical industry publication BioCentury explains[23]:

> "The Defense Research Advanced Projects Agency (DARPA) has taken risks where others wouldn't. Its pursuit of high-risk, high-reward technologies, combined with its mission-driven approach to managing projects is promising to pay off in the fight against COVID-19. DARPA was behind the creation of DNA and RNA vaccines, funding early R&D by Moderna Inc. and Inovio Pharmaceuticals Inc. at a time when the technologies were considered speculative by many scientists and investors."

In fact, a new study issued just a few weeks ago reported: "The unprecedented development of COVID-19 vaccines less than a year after discovery of this virus was enabled by more than $17 billion of research on vaccine technologies funded by NIH *prior to the pandemic*."[24] [emphasis added]

According to Kaiser Health News: "Basic research conducted … at the National Institutes of Health, Defense Department, and federally funded academic laboratories has been the essential ingredient in the rapid development of vaccines in response to COVID-19."[25]

Of course, the government invested an additional $18 billion through Operation Warp Speed and other programs.[26] As a result of all that taxpayer investment, The New York Times concluded: "A new method of developing vaccines was already waiting to be

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[21] Edwards, D. J. (2020, April 14). *New products alone are not enough. Pharma can do more to halt COVID-19*. Access to Medicine Foundation. https://accesstomedicinefoundation.org/media/uploads/downloads/5e95d85128fb9_ATMF_Viewpoint_Role_for_pharma_in_C-19_200414%20(1).pdf

[22] Johnson, C. Y. (2020, December 6). A gamble pays off in 'spectacular success': How the leading coronavirus vaccines made it to the finish line. *The Washington Post*. https://www.washingtonpost.com/health/2020/12/06/covid-vaccine-messenger-rna/

[23] Usdin, S. (2020, March 25). DARPA's gambles might have created the best hopes for stopping COVID-19. *BioCentury*. https://www.biocentury.com/article/304691/darpa-jump-started-technologies-behind-some-of-the-leading-covid-19-vaccine-and-antibody-hopes

[24] Bentley University. (2021, April 22). *COVID-19 vaccine development built on >$17 billion in NIH funding for vaccine technologies*. https://www.eurekalert.org/pub_releases/2021-04/bu-cvd042121.php

[25] Allen, A. (2020, November 18). Government-Funded Scientists Laid the Groundwork for Billion-Dollar Vaccines. *Kaiser Health News*. https://khn.org/news/vaccine-pioneers-basic-research-scientists-laid-groundwork-for-billion-dollar-pharma-products/

[26] Congressional Research Service. (2021, March 1). *Operation Warp Speed Contracts for COVID-19 Vaccines and Ancillary Vaccination Materials*. https://crsreports.congress.gov/product/pdf/IN/IN11560

tested … The government was willing to spend whatever it took, eliminating financial risks and … allowing mass production to begin even before trials were done."[27]

One noted industry expert, Jack Scannell, summed it up this way: "Before we pat the drug industry on the back too much, one has to recognize it got involved in this partly because the whole thing has been de-risked by government."[28]

4) Pharma's claims that patients will suffer an alarming loss of new drugs if anything is done to curb its unilateral pricing power isn't supported by the facts. The Congressional Budget Office found that we could cut pharma revenue by up to $1 trillion dollars over a 10-year period and lose only eight of 300 expected new drugs.[29] And many of those eight drugs would not be real losses at all because only 10 to 15 percent of new drugs that come to market actually represent true therapeutic advances.[30] Drug companies could be developing drugs that offer new hope for patients; instead, they focus resources on developing "me-too" drugs or on small changes that are designed to extend patent protection on existing products to keep generic competitors off the market. The loss of a few drugs each year will have minimal impact on the health of Americans.

5) Big Pharma threatens that patients will lose access to newly developed drugs. It points out that more drugs are available — and are available faster — in the United States than in other wealthy countries. It frequently references a white paper from the White House Council of Economic Advisers (CEA) to explain why: "Drug manufacturers usually pursue market access in the United States before other markets due to the higher prices in the United States."[31] The CEA could also have mentioned the other big reason drug companies file for approval first in the United States: It is the largest market in the world.[32-33]

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[27] LaFraniere, S., Thomas, K., Weiland, N., Gelles, D., Stolberg, S. G., & Grady, D. (2020, November 30). Politics, Science and the Remarkable Race for a Coronavirus Vaccine. *The New York Times*. https://www.nytimes.com/2020/11/21/us/politics/coronavirus-vaccine.html

[28] Neville, S., & Kuchler, H. (2020, November 27). Covid vaccines offer Big Pharma a chance of rehabilitation. *Financial Times*. https://www.ft.com/content/75029036-13f3-4ca2-8954-5a7207c0c3db

[29] Congressional Budget Office. (2019, October 11). *Effects of Drug Price Negotiation Stemming From Title 1 of H.R. 3, the Lower Drug Costs Now Act of 2019, on Spending and Revenues Related to Part D of Medicare*. https://www.cbo.gov/system/files/2019-10/hr3ltr.pdf

[30] Light, D. W., & Lexchin, J. R. (2012). Pharmaceutical research and development: what do we get for all that money?. *BMJ*, *345*. https://doi.org/10.1136/bmj.e4348

[31] The Council of Economic Advisers. (2018). *Reforming Biopharmaceutical Pricing at Home and Abroad*. https://trumpwhitehouse.archives.gov/wp-content/uploads/2017/11/CEA-Rx-White-Paper-Final2.pdf

[32] IQVIA. (2020, March 5). *Global Medicine Spending and Usage Trends*. https://www.iqvia.com/en/insights/the-iqvia-institute/reports/global-medicine-spending-and-usage-trends

[33] Association of Community Cancer Centers v. Alex M. Azar II. Civil Action No. CCB-20-3531 (2020). https://www.phrma.org/-/media/Project/PhRMA/PhRMA-Org/PhRMA-Org/PDF/P-R/PhRMA-Complaint-on-MFN-Rule-Filed-2020-12-04.pdf

> Given that U.S. prices for brand-name drugs are almost four times what many other wealthy nations pay, we can lower prices by a meaningful amount and still offer the highest prices by far in the largest market in the world, preserving the incentive to file first for approval in the United States.[2, 34]

There are other important policies in the U.S. drug pricing system that lead to more drugs being available here compared to other countries, none of which would be altered by lowering prices:

- Medicare must cover all drugs in six protected classes, which even PhRMA acknowledges ensures access to these drugs.[35-36]
- Medicare must cover at least two drugs in each class of drugs.[37]
- Medicaid must cover every drug offered by a manufacturer in the United States if the manufacturer agrees to give Medicaid a best-price guarantee.[38]

Pharma's threats to innovation and access don't hold up. It is clear that we can restore balance to have fair prices and profits and still get the innovation we need.

Equally important, we must remember that people can't afford existing drugs they need right now. More than 1.1 million Medicare patients could die over the next decade because they cannot afford to pay for their prescriptions. Medicare negotiation could lead to 94,000 fewer deaths every year. You could be responsible for those saved lives.[39]

Section IV. What We Can Do About It: Medicare Negotiation

Now Congress has the opportunity to act. With the reintroduction of *H.R. 3, the Elijah E Cummings Lower Drugs Costs Now Act,* and President Biden's strong support for allowing Medicare to negotiate directly with the drug corporations, Congress, and especially this chamber, can deliver on years of promises to take on high drug prices.

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[34] Mulcahy, A. W., Whaley, C., Tebeka, M. G., Schwam, D., Edenfield, N., & Becerra-Ornelas, A. U. (2021). *International Prescription Drug Price Comparisons*. RAND Corporation. https://www.rand.org/pubs/research_reports/RR2956.html

[35] Centers for Medicare & Medicaid Services. (2019, May 16). *Medicare Advantage and Part D Drug Pricing Final Rule (CMS-4180-F)*. https://www.cms.gov/newsroom/fact-sheets/medicare-advantage-and-part-d-drug-pricing-final-rule-cms-4180-f

[36] Powaleny, A. (2015, December 10). *Medicare Part D's six protected classes*. PhRMA. https://catalyst.phrma.org/medicare-part-d-six-protected-classes

[37] *What Medicare Part D drug plans cover*. (n.d.). CMS.gov. Retrieved May 3, 2021 from https://www.medicare.gov/drug-coverage-part-d/what-medicare-part-d-drug-plans-cover

[38] Kaiser Family Foundation. (2019, May 1). *Medicaid's Prescription Drug Benefit: Key Facts*. https://www.kff.org/medicaid/fact-sheet/medicaids-prescription-drug-benefit-key-facts/

[39] West Health. (2020, November 19). *New Study Predicts More Than 1.1 Million Deaths Among Medicare Recipients Due to the Inability to Afford Their Medications*. https://www.westhealth.org/press-release/study-predicts-1-million-deaths-due-to-high-cost-prescription-drugs/

H.R. 3 overturns the ban on direct Medicare negotiation and enables the secretary of Health and Human Services to negotiate for lower drug prices on the most expensive drugs in Medicare.

The legislation would bring relief not just to Medicare beneficiaries, but to all patients regardless of the type of insurance they have, by extending negotiated prices to the private sector.

Patients like Janet Bacon[40] would benefit. Janet relies on an inhaler priced at nearly $500 a month just to allow her to breathe. If prices continue to go up, she'll face the terrible choice of selling her home just to afford to stay alive.

H.R. 3 doesn't just allow Medicare to negotiate, it also includes other common-sense solutions to fix our drug pricing system that have enjoyed bipartisan support. It would penalize companies that hike prices faster than the rate of inflation. It would limit annual out-of-pocket costs for Medicare beneficiaries to $2,000 so patients like me wouldn't have to spend upwards of $18,000 a year for a single prescription. And I would point out, $2,000 is still a great deal of money for many Medicare beneficiaries whose median income is less than $30,000 per year.[41] One in four have incomes less than $17,000 per year. To lessen this burden, we suggest shoring up the low-income subsidy program so the lowest-income beneficiaries have no out-of-pocket costs.

These are straightforward proposals that would bring relief to millions of patients.

The legislation is estimated to save the federal government over $450 billion dollars.[42] We can put those savings to work in a variety of ways.

Big Pharma claims using these savings to address other critical needs is tantamount to using the industry as a piggy bank.[43] But in reality, it is pharma that has treated patients and taxpayers as piggy banks for years — raising prices at will to hit profit targets and trigger executive bonuses.[44]

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[40] Patients For Affordable Drugs Now. (2021, April 9). *My husband and I will be forced to sell our home*. https://patientsforaffordabledrugsnow.org/2021/04/09/janet-bacon/

[41] Koma, W., Neuman, T., Jacobson, G., & Smith, K. (2020, April 24). *Medicare Beneficiaries' Financial Security Before the Coronavirus Pandemic*. Kaiser Family Foundation. https://www.kff.org/medicare/issue-brief/medicare-beneficiaries-financial-security-before-the-coronavirus-pandemic/

[42] Congressional Budget Office. (2019, December 10). Budgetary Effects of H.R. 3, the Elijah E. Cummings Lower Drug Costs Now Act. https://www.cbo.gov/system/files/2019-12/hr3_complete.pdf

[43] Florko, N. (2021, April 13). PhRMA chief talks strategy — and he's surprisingly optimistic about drug pricing reform. *STAT*. https://www.statnews.com/2021/04/13/phrma-chief-talks-strategy/

[44] McAuliff, M. (2020, September 30). Sky-High Drug Prices Driven by Pharma Profits, House Dems Charge. *Kaiser Health News*. https://khn.org/news/sky-high-drug-prices-driven-by-pharma-profits-house-dems-charge/

Pharma is absolutely right about one thing: America does have other priorities. We can only spend a dollar once, and every dollar we send to pharma in unjustified profits — or "rents," as economists like to call them — is a precious dollar we don't have to tackle other urgent needs. It's a dollar we don't have to reduce health care disparities, provide coverage to the uninsured, or increase funding for research on new drugs based on public health needs instead of private profit needs.

That's why it is so important that H.R. 3 directs some of the savings to the NIH to fund the very innovation pharma claims will come to a halt if we rein in prices.

It's no surprise that Medicare negotiation is so popular, with 93 percent of Americans saying they support the policy.[10] That includes overwhelming majorities from both political parties.

Increasingly, employers support government intervention to limit the prices of drugs. In a recent survey of employers with more than 5,000 employees, almost 4 in 10 said they somewhat or strongly agreed that the government should negotiate lower drug prices; only three percent disagreed.[45]

H.R. 3 is a bipartisan solution with massive support. It is time for Congress to finally pass it into law.

While the headwaters of our drug pricing problems are the list prices set by drug corporations, there are other reforms needed in the supply chain. Pharmacy benefit managers (PBMs) are black boxes that cut secret rebate deals with manufacturers, and none of it is transparent.

It is simply wrong that patients like me don't know if the preferred drug on a PBM formulary is there because it is the best drug, because it is the least expensive drug among equally effective options, or because the PBM got a big, legal kickback from the manufacturer. Without transparency, it is impossible to know how much of a rebate is going to the PBM, to the insurer, to lower my premiums, or to reduce my out-of-pocket costs at the pharmacy counter. With more than $300 billion in drugs moving through PBMs, that is a bad way to run a railroad.[46] It's time for transparency to ensure PBMs are operating in the best interests of those they are supposed to serve — patients and consumers.

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[45] Claxton, G., Levitt, L., Gremminger, S., Kramer, B., & Rae, M. (2021, April 29). *How Corporate Executives View Rising Health Care Cost and the Role of Government*. Kaiser Family Foundation. https://www.kff.org/report-section/how-corporate-executives-view-rising-health-care-cost-and-the-role-of-government-findings/

[46] Pew Charitable Trusts. (2019, March 8). *The Prescription Drug Landscape, Explored*. https://www.pewtrusts.org/en/research-and-analysis/reports/2019/03/08/the-prescription-drug-landscape-explored

Section V. Conclusion

Let's be clear: Big Pharma is not fighting for the interest of patients — it's fighting to maintain its unilateral power to dictate prices of brand-name drugs. Recently, the head of the trade association PhRMA affirmed that fact in a moment of candor. He said his industry is "particularly adept at … rolling the tanks, if you will, to push back against policy proposals adverse to the industry's interests."[43]

So it's quite clear: You can choose a side. Stand with patients, consumers, and taxpayers for lower prices, or stand with pharma to protect "the industry's interests." Because let's be honest — that's what this fight is about.

Of course, Big Pharma wants to disguise that truth. Instead, it blames others and distracts attention from its central role in making drugs unaffordable.

And it tries to scare us by saying that if we don't bend to its will, we won't get the drugs we need for the future. It poses questions like: How much would you pay to save a life?

And that's easy. When it's you or someone you love, the answer is anything.

But that's the wrong question. We should be asking: *How do we restore balance to ensure we get the innovation we need at prices we can afford?*

One of our patients is Marcus LaCour from Ohio.[47] He's a husband, a father, and a minister. He is also a person with type 1 diabetes. Since he was diagnosed in high school, struggling to afford insulin has been a pattern in his life. He's been forced to rely on samples from his doctor, ration his insulin, or simply go without. In some of his hardest times, he rationed his insulin while his wife skipped meals to help pay for it. This should not happen in America.

I feel incredibly grateful to spend my retirement fighting so that people like Marcus can one day enjoy theirs.

All of you hold the power to fix this broken system. It's time to enact comprehensive reforms and lower prescription drug prices.

Thank you.

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[47] Patients For Affordable Drugs. (2021, April 5). *I've been forced to ration my insulin or simply go without*. https://patientsforaffordabledrugs.org/2021/04/05/marcus-lacour-innovation/

Chairman DESAULNIER. Thank you Mr. Mitchell. Next up is Dr. Holtz-Eakin. Doctor the floor is yours.

STATEMENT OF DOUGLAS HOLTZ-EAKIN, PRESIDENT, AMERICAN ACTION FORUM

Mr. HOLTZ-EAKIN. Well, thank you, Chairman DeSaulnier, Ranking Member Allen and Members of the Subcommittee. It's a privilege to be here today to discuss this very important topic. I'd like to make three brief points, and then I look forward to the opportunity to answer your questions.

Point No. 1 is that there's no evidence of a broad-based pervasive drug pricing problem in the United States that would merit a

sweeping one size fits all solution. As I laid out in my written testimony if you look at a variety of measures of price, list price, net price, cost out of pocket to a beneficiary, look across beneficiaries, and look across drugs.

On the whole there is no real evidence of a sharp uptick in prices that would merit a strong policy response. There are in fact select individuals and drugs for which there is a severe financial problem, but that suggests the right response is a targeted solution in those circumstances, and that the Committee should be focused on those.

So this data really do suggest that you focus on increasing the supply. Any time we have a high price you need to look at the supply of drugs for those targeted situations. Point No. 2 is that the provisions in Title I of H.R. 3 are hardly a negotiation, and are in fact collectively a threat to the dynamic, innovative eco-system of the U.S. pharmaceutical industry.

It's not a negotiation. The Congressional Budget Office has repeatedly beginning with my tenure informed the Congress that giving the secretary the power to negotiate would produce little, if any, in the way of budgetary savings for the Federal Government. And the reason is quite simple the secretary doesn't have at his or he disposal a formulary or other lever to negotiate effectively with funds from the manufacturers.

The plans and PBMs that negotiate in Part D do have those tools and negotiate very effectively. The difference between those findings and those CBO findings for H.R. 3 thus lie in the tax provisions in H.R. 3 and the international reference prices the average international market price provisions of that legislation.

The tax provisions are real simple. There's a draconian 95 percent revenue tax that is simply a threat to the existence of the drug on the domestic market, and is tantamount to asking the company to withdraw that drug. This raises the fundamental issue of access to therapies that is so important to Americans.

And that tax is there to ration those therapies. The average international market price is an arbitrary price ceiling being set by the Federal Government. It's not a negotiated or market-based price. And it also threatens access to drugs. If you look at the referenced countries underneath that averaging index market price, you do find less access to pharmaceuticals.

They're on the market slower, they're on in the U.S. and in about 4 months they're on much slower in those other countries. Almost 90 percent of brand name drugs will be on the U.S. market. At best 60 percent will be on in referenced countries. Sometimes it's only a third.

And so there's a fundamental tradeoff there whether you're going to pay in the form of financial costs, or in lack of access to important therapies.

Point No. 3 is that if you want to do something on drug prices I would propose instead that you look at the reforms to the Medicare Part D benefit in H.R. 19. Medicare Part D has been enormously successful settling program as I lay out in my testimony.

However, you could sharpen the incentives for better negotiation by making manufacturers and plans liable for more of the costs in the catastrophic region, and thus reduce the incentive to have high-priced drugs that push individuals into that region. You can save

the taxpayer an increasing bill that comes with the reassurance item in the catastrophic region, and by capping out of pockets you can improve the benefit for beneficiaries in an already popular and successful benefit will be even more suitable for American seniors.

So that I think is a better route to go. Part D counts for 25 percent of drug spending in the United States. It's a very important lever for improving private negotiations and market incentives to deliver pharmaceuticals at a reasonable cost. So that I think is a route you ought to consider and steer away from Title I which I think will in the end do more damage than good.

So I thank you for the chance to be here and I look forward to your questions.

[The prepared statement of Mr. Holtz-Eakin follows:]

PREPARED STATEMENT OF DOUGLAS HOLTZ-EAKIN

Testimony Regarding:
Lower Drug Costs Now: Expanding Access to Affordable Health Care

U.S. House of Representatives
Committee on Education and Labor
Subcommittee on Health, Employment, Labor, and Pensions

Douglas Holtz-Eakin, President*
American Action Forum

May 5, 2021

*The views expressed here are my own and do not represent the position of the American Action Forum. I am indebted to my colleagues Tara O'Neill Hayes and Christopher Holt for their continued efforts to educate on these issues.

Chairman DeSaulnier, Ranking Member Allen, and members of the Subcommittee, thank you for the opportunity to testify today on the matter of drug prices. I hope to make three basic points:

1. The term "rising drug costs" is riddled with ambiguity; list prices, net prices, out-of-pocket prices, development costs, and total spending on drugs have displayed very different patterns over time.

2. The provisions of Title I of H.R. 3 are far from a government "negotiation" and are a threat to the dynamic ecosystem that drives U.S. pharmacological innovation.

3. A superior approach would be the reforms to Medicare Part D in H.R. 19, a superior version of the reform in Title III of H.R. 3.

Let me discuss these further.

Introduction

Over the past several years, the public's attention has increasingly been focused on the cost of health care, and specifically the contribution of prescription medications to those costs. Policymakers, however, should first clearly identify the actual problem they're trying to address.

Identifying the Problem: Patterns in Drug Costs

There is little consensus in the term "rising drug costs," making it difficult to determine if there is an actual policy problem, its size, or its scope. The first step in identifying whether there is a problem is to differentiate between prices, costs, and spending, which are related but not identical.

For example, "rising drug costs" might refer to a narrow definition focused on the sales prices (or "list price") set by drug developers and manufacturers. Alternatively, the problem might not be with all drugs, but instead the high prices of some drugs. Finally, the problem may be the increasing cost of prescription drugs borne by individuals at the pharmacy counter, which has resulted from an increase in high-deductible health plans and greater use of co-insurance, rather than flat co-pays.[1]

Rising drug costs could also mean an increase in overall prescription drug expenditures, whether in dollar figures or as a percentage of National Health Expenditures (NHE). Because spending is a function of both price and quantity, this could result from increased utilization due to rising national reliance on prescription drugs or broader access to them.

Pharmaceuticals as a Share of National Health Expenditures

The first important fact to consider is that prescription drug spending as a percent of NHE has remained steady at about 10 percent since 2000, the same percentage it was in 1960. There was a dip in prescription drug spending as a share of NHE in the years between 1960 and 1980, as advances in technology and expanded insurance coverage of hospital visits contributed to a shift in NHE towards hospital stays.[2] In the 1980s, that trend began to reverse as new pharmaceuticals became widely available for the treatment of many of the most prevalent diseases in American society. The availability of advanced pharmacological treatments is highly correlated with reduced expenditures for hospitals and other health professionals.[3] In fact, the Congressional Budget Office estimated in 2019 that increased use of prescription drugs would lead to twice as much in cost savings on hospital and physician services as it would increase costs on pharmaceuticals.[4] As pharmaceutical growth began to level out to roughly the same levels as the 1960s, so did other NHE categories.[5] Viewed from this national perspective, there appears to be little empirical support for a perceived radical rise in drug spending in the data, although national averages can mask the variance among subpopulations and the most current NHE data is more than a year old.

Drivers of Drug Spending

To the extent that drug expenditures are increasing or will begin to increase in the near future, a key factor is utilization. Annual growth in pharmaceutical spending in February 2020 was 7.9 percent,[6] but annual pharmaceutical price growth was only 2.4 percent.[7] On a per capita basis, real net spending has grown by only 1 percent between 2007 and 2017 and actually declined by 2.2 percent in 2017.[8]

Still, Americans are getting older, living longer, and are increasingly burdened with chronic disease. As of this year, 60 percent of the United States' adult population had been diagnosed with at least one chronic health condition, and 40 percent had two or more chronic conditions.[9] Managing these chronic conditions is an expensive proposition that relies primarily on medication. Eighty-six percent of all health care spending is for patients with one or more chronic disease; 98 percent of Medicare and 83 percent of Medicaid spending goes toward providing care for the chronically ill.[10, 11] Specifically, over 75 percent of U.S. health care spending goes toward treatment of chronic disease.[12] As these trends continue, the financial burden of maintaining a high quality of life with chronic conditions will inevitably disproportionately increase the growth of pharmaceutical health care spending.

Drivers of Drug Prices

Developing new treatments is an expensive prospect in terms of both capital and time. A Tufts University study in 2016 found that the average cost for each drug successfully brought to the market is nearly $2.9 billion.[13] Data from the Organisation for Economic Co-operation and Development also show that the amount of spending per new drug approved has been growing for decades.[14] It takes an average of 15 years from the time a drug

developer first begins testing a new formula until the Food and Drug Administration (FDA) approves it.[15] Only 1 in 1,000 drug formulas will ever enter pre-clinical testing, and of those, roughly 8 percent will ultimately receive FDA approval.[16]

Additionally, the last decade has seen a significant shift toward the use of "specialty drugs." While there is no precise definition of a specialty drug, this term typically refers to drugs with at least one of the following characteristics: requires special handling, must be administered by a doctor, requires patient monitoring or follow-up care, or is used to treat complex, chronic conditions.[17] As a result, these drugs tend to be quite expensive. In fact, by 2016, about half of the top 80 most expensive drugs nationally were specialty drugs, and that number is increasing annually.[18] In 2010, the United States spent just over $11.5 billion on the top 25 specialty drugs. By 2018, net spending on specialty medicines reached $170 billion, accounting for 49.5 percent of all expenditures on medicines, despite accounting for just 2.2 percent of the volume.[19] Because specialty drugs are often more expensive to develop and typically treat small patient populations with very specific and otherwise untreatable diseases, they tend to have higher prices. Over time, the cost of new specialty drugs per patient will likely continue to be higher as the target population for each new drug will grow smaller with the development of treatments for less common diseases.

List Versus Net Prices

An important aspect of the discussion is the difference between list price and net price. List prices for brand-name drugs, on average, increased between 5.2 and 9.3 percent between 2015 and 2019, yet the average net price of these drugs has grown between 0.3 and 2.9 percent, with the trend being flat or downward sloping.[20] In fact, price growth for prescription drugs over the course of 2018 was the lowest growth rate since 2013, and even dipped into negative territory between December 2017 and early 2018.[21] So while the average list price of brand name drugs rose 69 percent between 2010 and 2019, average out-of-pocket (OOP) costs for those drugs declined from $27.72 in 2015 to $26.25 in 2019.[22]Generic list prices have declined, on average, during this time period, and insured patient OOP costs have remained relatively unchanged.[23] The increasing difference between list and net price points to the growing use of discounts and rebates. Understanding the role of these incentives in price determination is an area worthy of careful consideration to ensure resources are being allocated as desired.

Out-of-Pocket Prices

From a patient perspective, many anecdotally report that OOP costs are climbing and the increased frequency of high-deductible health insurance plans is cited as the reason. But the data show that average patient OOP costs at the pharmacy counter have actually declined since 2013. Roughly 30 percent of all medicines were available in 2019 for zero OOP costs, and 90 percent were available for $20 or less, with the average OOP cost for insured patients equaling $10.67. Only 1.1 percent of prescriptions filled had a co-pay of more than $125. Uninsured patients have seen their OOP costs increase 38 percent, though only 29 percent of patients paying cash face costs greater $125.[24]

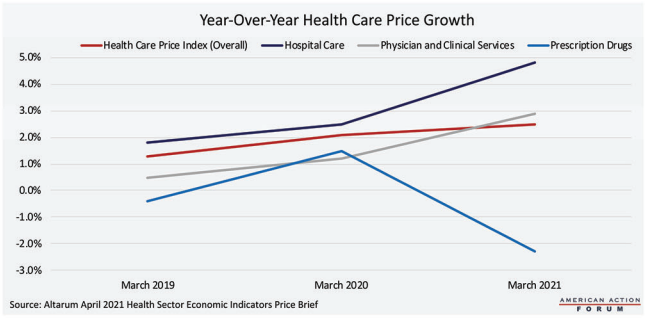
For the small share of very costly drugs, the expense adds up fast: 3.4 million prescriptions (0.1 percent of all prescriptions filled in 2017) had an OOP cost of more than $500, with an average cost of $1,502; total OOP expenditures for these drugs was $5.2 billion.[25] In 2019, 9.9 percent of patients paid more than $500 in annual OOP costs, including 2.3 percent who paid more than $1,500.[26] Seniors enrolled in Medicare Part D are most likely to have such high OOP costs, reflecting both their greater likelihood to take prescription medicines, as well as the program's benefit design, namely the lack of an OOP cap. It is likely also true that a number of prescriptions that would have cost at least that much were never filled because the patient simply could not afford it (or chose not to spend the money). The abandonment rate for brand-name drugs reached 21 percent in 2017.[27]

Recent Developments in Drug Prices

Over the past three years, overall health care prices have gradually increased, rising 1.3 percent between March 2018 and March 2019 and 2.5 percent from March 2020 to March of this year. There are, however, noticeably different rates of growth across the various health care product and service lines.

For example, hospital prices grew 1.8 percent and 2.5 percent year-over-year by March 2019 and March 2020, respectively, before jumping 4.8 percent by March 2021. Prices for physician and clinical services followed the same trend, but at a slower rate, rising 0.5 percent, 1.2 percent, and 2.9 percent in each of the past three years.

Prescription drug prices, on the other hand, have declined in two of the past three years: down 0.4 percent from March 2018 to 2019, rising just 1.5 percent by March 2020, and declining 2.3 percent by March 2021. In fact, March was the sixth straight month that prescription drug prices showed a year-over-year decline. These figures indicate that health care price growth is not being driven by prescription drug prices, but rather by ever-rising hospital prices, primarily, particularly given that hospital care accounts for nearly 40 percent of all health care expenditures while prescription drugs account for less than 20 percent.



Title I of H.R. 3
Title I of H.R. 3 would require the Secretary of Health and Human Services (HHS) to enter into a binding negotiation process with the manufacturers of at least 25 branded drugs, and up to 250 drugs, each year to set the Maximum Fair Price (MFP) for each drug for all third-party payers. As a starting point for the negotiation, the Secretary would establish a ceiling price of 120 percent of the volume-weighted average price of the drug in Australia, Canada, France, Germany, Japan, and the United Kingdom, or the Average International Market (AIM) price. Once the negotiations conclude and the new MFP is established, manufacturers would be prohibited from increasing their price above the rate of inflation. If the Secretary concludes that a manufacturer is not negotiating in good faith, the drug can be subjected to a tax equal to 95 percent of its revenue, in effect guaranteeing that the drug would be withdrawn from the domestic market.

It is worth emphasizing the sweeping nature of the proposed reforms. The MFP does not just apply to Medicare Part D, or Medicare as a whole, or even just federal programs. It would apply to all third-party payers, including plans operating under the Employee Retirement Income Security Act (ERISA).

The provisions are a uniquely undesirable combination of government "negotiation" and international reference pricing. Let us discuss each in turn.

Government Negotiation

Government negotiation has figured strongly in the debate over the structure of the Medicare Part D program. Direct negotiation by the Secretary of HHS has been expressly forbidden in the Part D statute. Yet the program nevertheless sees aggressive negotiation

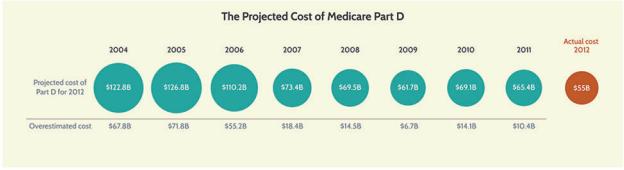
over the prices of medications between Part D plan sponsors and drug manufacturers. This competitive process is the key factor in the program's success to date.

Today, Part D beneficiaries have access to 27 different plans, on average, enabling individuals to choose a plan that is tailored to their needs.[28] Because there are a number of plan options for beneficiaries, individual plans have the ability to use preferential tiering strategies to negotiate discounts for specific drugs. If a beneficiary requires or desires a specific medication that is not on the preferred formulary (or covered at all) for one plan, they can choose to sign up for a different plan that provides the medication at a more desirable price.

If the government, however, were to seek to negotiate the prices of specific drugs, the system would break down. Plans have leverage to drive discounts because they can restrict or deny access to specific medications or offer the medication in ways that make it more desirable to their beneficiaries. For the federal government to undertake this kind of negotiation, there would need to be a single federal formulary. In other words, the Secretary would have to be willing to say no to many treatments on behalf of all beneficiaries in order to drive discounts system-wide. Beneficiaries' choices would drop from 27 plans to 1. Further, beneficiaries would no longer be able to shop for the plan that is best for them; rather, they would have to simply hope the government was able to negotiate a good deal for the drug(s) they need. Policymakers and the American public have long been reticent to make that trade off.

The Congressional Budget Office (CBO) has repeatedly held that in absence of a willingness to deny coverage for specific medications, the Secretary would not have the leverage necessary to drive any savings to the Part D program.[29] In short, given these constraints, direct negotiation of drug prices by the secretary would not work. H.R. 3 "solves" this problem by giving the Secretary access to a draconian tax that would effectively preclude access to the drug. This leverage, not negotiation, is the source of any projected savings.

The current reliance on genuine, private negotiation has worked incredibly well. As demonstrated in the following infographic, total program expenditures came in far lower than initial CBO projections. Part D's 10-year cost (starting in 2006) was projected in 2004 to be $957.3 billion, after the Medicare Modernization Act was passed but before the program started. By 2011, the combination of five years of actual data and five years of projections totaled $499.4 billion, for a cost under-run of $457.9 billion, or about 48 percent. The last CBO forecast for 2012 Part D spending made prior to implementation was in 2005, and the projected 2012 spending in that year was $126.8 billion. After the bids came in for 2006, the 2012 forecast was reduced to $110.2 billion. In all but one of the next six years, the forecast for 2012 was reduced further. The actual amount was $55.0 billion – about 57 percent lower than the original pre-implementation forecast.[30]



International Reference Pricing

As a starting point to the process outlined in Title I, the Secretary would establish a ceiling price of 120 percent of the volume-weighted average price of the drug in Australia, Canada, France, Germany, Japan, and the United Kingdom, or the Average International Market (AIM) price. This is a form of international reference pricing, also employed in the Trump Administration's regulatory efforts to set an International Pricing Index (IPI) or a Most-Favored Nation (MFN) price in Medicare.

While the objective of reducing the cost of drugs and increasing Americans' access to necessary medicines is laudable, such a policy could result in significant undesirable repercussions. The most likely consequences are restricted access to existing medicines and reduced innovation for future advancements and new medicines; cost-shifting to the private sector insurance markets; an undermining of the administration's goal to move to value-based payments; and harm to U.S. trade objectives.

To see the implications, consider the IPI proposal. The 14 countries that CMS proposed referencing in the IPI model, on average, have access to only 48 percent of the new drugs developed in the past eight years, and it took an average of 16 months after their initial global launch for those drugs to become available in those 14 countries. The United States, on the other hand, has gained access to 89 percent of new medicines within three months.[31]

Adopting the non-market prices of other countries, and thus the punitive and authoritative policies used to obtain those prices, will likely also mean adopting for American patients similar levels of restricted access to new medicines as experienced in other countries. Worse yet, this demo may result in new medicines never being developed in the first place. Operation Warp Speed, undertaken by the Trump Administration, has been widely praised for accelerating the development COVID-19 vaccinees and therapies, saving lives and allowing for a faster economic recovery. But while Operation Warp Speed involved substantial federal investment, the effort was only possible because of years of industry research and development prior to the pandemic.[32] In a world where policies like those in H.R. 3 had been in place in preceding years, research may not have progressed far enough for an effort like Operation Warp Speed to be effective.

Americans highly value their access to and choice of new treatment options. The reduced innovation that will likely occur as a consequence of the reduced manufacturer revenues that will result from this model will have significant ramifications. Further, referencing the prices paid for drugs in countries that do not adequately reflect the value of medicines is inconsistent with adopting a value-based payment system.

Finally, this model will undermine American trade policy, which may have repercussions far beyond the pharmaceutical industry. The United States should instead work to strengthen intellectual property rights in other countries and fight compulsory licensing in trade agreements to end the coercive practices that allow countries to force manufacturers to provide their drug for less than it's worth; this is the only way to get other countries to pay more so that we may hopefully pay less without risking reduced innovation.

Reforms to Medicare Part D

Title III of H.R. 3 contains reforms to Medicare Part D. A reform of this type would be a valuable improvement in Part D, if the parameters are set appropriately, and would place downward pressure on drug prices more generally.

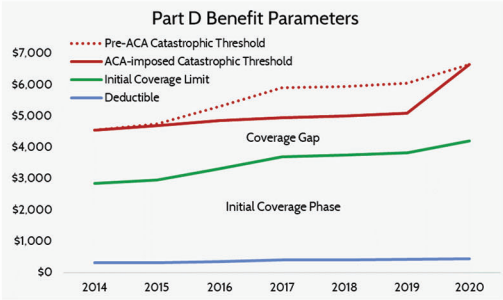
Reasons for Reform

One reason for reform is to rein in rising reinsurance costs. At the program's outset in 2006, reinsurance costs accounted for 26 percent of the government's overall Medicare Part D subsidy. By 2019, reinsurance costs had climbed to 80 percent of the program's basic subsidy, at $46.3 billion.[33] This shift has dramatically increased taxpayers' financial exposure, putting the program on an unsustainable path. Between 2007 and 2017, premium subsidies decreased by 1.8 percent per year while reinsurance payments increased by 16.7 percent per year, on average.[34]

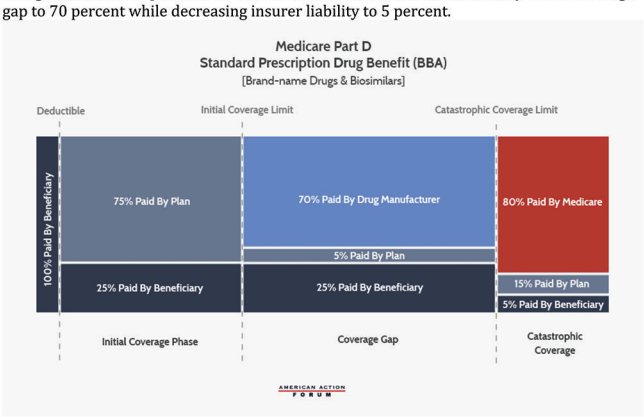
While rising drug spending itself is, of course, partially to blame for the increasing reinsurance costs, structural changes made to the program are also significant contributors. The Patient Protection and Affordable Care Act included two provisions that made significant changes to the Part D program: the Coverage Gap Discount Program—otherwise known as "closing the coverage gap" (or the "donut hole")—and a temporary slowing of the catastrophic threshold's growth rate.

The Coverage Gap Discount Program required drug manufacturers to pay 50 percent of the costs of any drug a beneficiary takes while he or she is in the coverage-gap portion of the benefit phases. These manufacturer payments count toward the beneficiary's true out-of-pocket (TrOOP) cost calculation, which in turn determines when the beneficiary moves from one phase of the benefit to the next. Including manufacturer's payments in this calculation results in beneficiaries moving through the benefit phases and into the catastrophic phase much more quickly than they otherwise would.

The temporary slowdown in the growth rate of the catastrophic threshold also led to more beneficiaries reaching the catastrophic phase and doing so after less spending than they would have absent this change, as can be seen below.



The growth rate of the catastrophic coverage threshold has now returned to normal, but a provision included in the Balanced Budget Act of 2018 (BBA) exacerbates the problem of rising reinsurance expenditures: The BBA increased manufacturer liability in the coverage gap to 70 percent while decreasing insurer liability to 5 percent.



The increase in manufacturer liability exacerbates the existing TrOOP problem. This change resulted in an unprecedented increase in the number of beneficiaries reaching the catastrophic phase, driven largely by beneficiaries not receiving the low-income subsidy (LIS). In 2019, 4.3 million individuals reached the catastrophic phase, with a 33 percent increase among non-LIS beneficiaries from the year prior.[35]

Further, the decrease in insurer liability significantly diminishes insurers' incentive to control costs for high-cost enrollees. Insurers in 2019 were at risk for only 38.8 percent of the benefit costs, down from the expected 75 percent rate in 2007.[36] In fact, the BBA reduced insurer liability so substantially that for a beneficiary who reaches the catastrophic threshold in 2020 taking exclusively brand-name or biosimilar drugs, the insurer's cost at this point ($2,974) is about $700 less than the drug manufacturer's ($3,698).

A second issue is the financial burden on high-cost enrollees. The financial burden for enrollees reaching the catastrophic phase can be quite significant. In 2010, 2.4 million beneficiaries reached the catastrophic phase, and 33,000 beneficiaries did so after filling a single prescription.[37] In 2019, more than 4.3 million beneficiaries reached the catastrophic phase and more than 483,000 did so after a single prescription fill, up more than 100,000 just two years prior.[38] These beneficiaries had average spending of $26,482.[39] Beneficiaries who do not receive the LIS are responsible for 5 percent of all costs in the catastrophic phase, meaning the average non-LIS high-cost enrollee incurred more than $2,100 in OOP costs in 2019, and that figure will increase each year, particularly as more expensive specialty medicines are developed. As of 2019, specialty drugs account for 25 percent of Part D spending, approximately four times more than in 2010.[40]

The larger reason for reform is the perverse incentives for high-priced drugs. The structure of the current benefit design, as well as the pricing incentives in the broader drug market, encourage insurers to prefer coverage of high-price, high-rebate drugs, a preference that also leads to more beneficiaries reaching the catastrophic phase. Because of the BBA's changes and the insurers' varying liability for brand-name products (5 percent) versus generics (75 percent), it is now in insurers' financial interest to prefer a brand-name drug over a generic unless the generic is more than 15 times less expensive than the brand-name product.[41] This incentive may depress demand for generics, which could discourage generic development, harming the broader market. Further, pharmacy benefit managers (PBMs)—working on insurers' behalf—have created a business model in which their revenue is largely tied to the amount of rebates they are able to obtain from drug manufacturers.[42] Drug manufacturers provide greater rebates in order to secure preferred status on a drug plan's formulary, which increases sales of the preferred drug. The easiest way to provide a larger rebate is to raise the price of the drug before the rebate (list price). Insurers benefit from greater rebates because these rebates, most often paid after the point of sale, can be used to reduce next year's premiums—the most important factor beneficiaries consider when deciding in which plan to enroll.

The mandatory discounts that Part D requires of drug manufacturers in the coverage gap also encourage higher drug prices. Because the coverage gap is in the middle of the benefit structure, only a finite amount of spending is subject to the discount, resulting in a maximum discount that manufacturers will owe.[43] Under current law, the maximum discount a drug manufacturer will have to pay in 2022 is $4,136. Any manufacturer with a drug costing $10,399 or more will have to pay this discount. For a drug costing $10,399, this discount represents a discount of 40 percent. For a drug costing $50,000, this maximum discount will only equal 8.3 percent of the drug's price. Thus, the current structure is more punitive to manufacturers of lower-cost drugs and encourages higher prices.

Further, patients who pay coinsurance based on the list price are substantially worse off when list prices rise. Also, because the rebates are used to reduce premiums for everyone, rather than simply reducing the high OOP costs paid by the patients taking the drug for which the rebate was paid, the beneficiaries with higher costs end up subsidizing those with lower costs—the opposite of how insurance is supposed to work. Taxpayers, who subsidize 75 percent of the program, are also worse off when program costs increase. Thus, higher-price, high-rebate drugs are beneficial to each of the industry stakeholders but increase costs for both patients and taxpayers.

Redesigning the Benefit to Realign Incentives

One possible reform to address these various problems would involve reconfiguring the liabilities within the Part D structure. These changes include placing a true cap on beneficiary OOP expenditures, eliminating the coverage gap phase entirely and instead requiring drug manufacturers to pay rebates during the catastrophic phase, reducing the federal government's reinsurance rate, and increasing plans' liability in the catastrophic phase.[44]

Requiring drug manufacturers to pay discounts in the catastrophic phase ensures that the amount they owe increases along with the drug's price, discouraging both high launch prices and price increases. The current structure—which caps manufacturer liability as described above—results in the mandatory discount being more punitive for lower-cost drugs, thus encouraging higher prices.

Increasing insurer's liability throughout the benefit, and particularly in the catastrophic phase, increases insurers' incentive to control costs and reduce the use of high-cost, high-rebate drugs.

Reducing the government's reinsurance liability reduces taxpayers' exposure to rising costs.

Establishing an OOP cap provides beneficiaries with financial protection, assuring them they will not face open-ended OOP costs. More than a million beneficiaries could save hundreds or thousands of dollars each year.

While Title III of H.R. would make all of these changes, analysis by the American Action Forum finds that the specific parameters —the level of the OOP cap, the discount rate required of drug manufacturers, and the liability placed on insurers—will not yield optimal outcomes.[45] The parameters established by H.R. 19—particularly the slightly higher OOP cap, reduced beneficiary cost-sharing below the OOP cap, and uniform insurer liability for generics and brand-name medicines—are more likely to do so.

The lower OOP cap established in H.R. 3 should yield greater OOP savings for the minority of beneficiaries who reach the cap, but it will also result in greater federal reinsurance costs, relative to H.R. 19. Because most beneficiaries never reach $2,000 in OOP spending, more will benefit from the reduced cost-sharing below the catastrophic phase provided by H.R. 19, yielding greater total OOP savings for beneficiaries.

Further, because insurer liability under H.R. 3—both above and below the catastrophic phase—would be lower for brand-name drugs than generics, this legislation would maintain the current perverse incentives for plans to favor more expensive brand-name drugs over cheaper generic options, which increases costs for other stakeholders. H.R. 19, on the other hand, provides a uniform liability for insurers regardless of the type of drug, eliminating this preference, which should encourage use of less expensive medicines.

Conclusion

Fundamentally, there is no broad prescription-drug pricing crisis. Indeed, in most instances, things are working just fine. Rather, what we face are more nuanced challenges. For example, the price of specialty drugs and biologics, which are expensive to develop and manufacture and frequently treat a limited population, are very high. In these instances, particularly with oncology drugs, it is important to make sure that the cost of the treatments correlates to the value. Remember that the goal should not be low cost, but rather high value. It is easy to have low-cost drugs; they, however, may not do much good. Conversely, it might make sense to spend more for a drug if its therapeutic benefits are high enough.

The policies contained in Title I of H.R. 3 will stifle the kind of innovation that has made the United States the location of the most advanced medical therapy on the globe. As CBO dryly put it: "The lower prices under the bill would immediately lower current and expected future revenues for drug manufacturers, change manufacturers' incentives, and have broad effects on the drug market."

In contrast, the reforms of Medicare Part D are similar to the proposal first put forward by the American Action Forum in 2018 and were included in bills from all sides in both the House and the Senate. As noted above, the preferred formulation is contained in H.R. 19. The basic ideas are to provide beneficiaries an out-of-pocket cap, reduce the government's reinsurance liability in the catastrophic phase, and requiring drug manufacturers to pay a share of the costs incurred in the catastrophic phase. The latter would increase the incentive for the manufacturer and insurer to negotiate prices that keep people out of the

catastrophic phase of their insurance policy. If something "must" be done about drug prices, this would appear to be the most promising legislative route.

In either event, the Congressional Budget Office has indicated that the reforms will generate budget savings. Those savings should not be used to finance a broad expansion of government spending. Instead, they out to be used to extend the financial life of the Medicare program, or reduce overall spending.

Notes

[1] https://www.healthsystemtracker.org/brief/payments-for-cost-sharing-increasing-rapidly-over-time/
[2] https://www.americanactionforum.org/research/understanding-pharmaceutical-drug-costs/
[3] https://www.americanactionforum.org/research/understanding-pharmaceutical-drug-costs/
[4] https://www.cbo.gov/system/files/2019-05/55151-SupplementalMaterial.pdf
[5] https://www.cms.gov/Research-Statistics-Data-and-Systems/Statistics-Trends-and-Reports/NationalHealthExpendData/index.html.
[6] https://altarum.org/sites/default/files/uploaded-publication-files/SHSS-Spending-Brief_April_2021.pdf
[7] https://altarum.org/sites/default/files/uploaded-publication-files/SHSS-Price-Brief_March_2021.pdf
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[24] https://www.iqvia.com/insights/the-iqvia-institute/reports/medicine-spending-and-affordability-in-the-us
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[33] http://www.medpac.gov/docs/default-source/default-document-library/ma_vip_public_jan_2020.pdf?sfvrsn=0
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[35] http://www.medpac.gov/docs/default-source/reports/mar21_medpac_report_ch13_sec.pdf?sfvrsn=0
[36] http://www.medpac.gov/docs/default-source/reports/mar21_medpac_report_ch13_sec.pdf?sfvrsn=0
[37] http://www.medpac.gov/docs/default-source/reports/mar19_medpac_ch14_sec.pdf?sfvrsn=0
[38] http://www.medpac.gov/docs/default-source/data-book/july2020_databook_sec10_sec.pdf?sfvrsn=0

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[39] http://www.medpac.gov/docs/default-source/reports/mar21_medpac_report_ch13_sec.pdf?sfvrsn=0
[40] http://www.medpac.gov/docs/default-source/reports/mar19_medpac_ch14_sec.pdf?sfvrsn=0
[41] For low-income subsidy (LIS) enrollees, the distortion is even worse because plans have no liability for such beneficiaries in the coverage gap.
[42] https://www.americanactionforum.org/research/primer-prescription-drug-prices-discounts-fees-effects-part-d/
[43] https://www.americanactionforum.org/insight/the-impact-of-shifting-rebates-to-catastrophic-coverage-in-medicare-part-d/
[44] https://www.americanactionforum.org/research/redesigning-medicare-part-d-realign-incentives-1/
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Chairman DESAULNIER. Appreciate your testimony. Finally, we'll hear from Mr. Isasi. The floor is yours.

STATEMENT OF FREDERICK ISASI, JD, MPH, EXECUTIVE DIRECTOR, FAMILIES USA

Mr. ISASI. Thank you so much Chairman DeSaulnier, Ranking Member Allen, Chairman Scott, Ranking Member Foxx and all the Members of the Committee. Thank you for the opportunity to testify today.

I'm Frederick Isasi, the Executive Director of Families USA. We're a non-partisan, non-profit that for over 40 years has served as one of the leading national voices for health care consumers, both in Washington, DC and on the State level.

Thank you very much for holding this hearing on lowering drug costs. What an incredibly important issue, and one that is so wildly popular with your constituents. Stopping prescription drug abuse is the No. 1 health care issues for voters across this Nation, and despite all of the division in our Nation's politics right now, Americans are united on this issue.

The vast majority of Americans want action right now on this issue, and the majority of others in both parties want the government to get in there and negotiate prices. As you've heard millions of Americans live with the fear of not being able to afford their prescriptions, and one-third of Americans are not taking their prescriptions because they are too expensive.

Of these, more than two-third are engaging the terrible gamble of either skipping doses, or cutting their pills in half. Year after year prescription drug companies launch drugs here in the U.S., and as you've heard charge three or four times more than other countries, and then in their greed these raise these outrageous prices much faster than either our paychecks or inflation.

In fact a record setting 900 drugs have seen abusive increases in prices since January of this year. The American people need relief. This is happening in red states and blue states for families in Richmond, California or in Savannah, Georgia.

The drug industry makes a lot of false arguments, and at its core the problem of out of control drug prices is very simple. Congress created a system that provides government granted monopoly to drug makers, and many within the industry are abusing these Federal laws.

Let me explain what I mean. Over time so much of the industry's focus has shifted from creating innovative drugs that can save lives, to doubling down on high-powered lawyers to find loopholes, sue competitors, and generally abuse the spirit in which Federal prescription drug laws were created.

That adds up to a crisis for families and hundreds of billions of dollars in waste. We at Families USA are strongly supportive of H.R. 3 the Elijah Cummings Lower Drug Cost Act which represents a critical step in addressing the crisis of prescription drug costs.

It represents bold and wildly popular action that would allow the government to defend our families and negotiate directly with drug manufacturers to curb abusive prices. The bill uses savings and reduced drug costs to invest in research and development for new cures, as well as reducing out of pocket costs for Medicare beneficiaries.

It also could support much needed improvements in the Medicare program such as dental, hearing and vision benefits, and support for low-income Medicare beneficiaries. So what will the fate of this important and wildly popular legislation be? On the other side of this fight is one of the most profitable and concentrated industries in the world with revenues in excess of a trillion dollars.

And half of its profits are generated in the U.S. and Canada alone. An industry spending at least $133 million just to lobby Congress, all of you, with over 800 lobbyists in DC. You are all under tremendous pressure.

Let me tell you about a remarkable woman we have met through our work at Families USA who perhaps can steady your resolve and get this legislation enacted. Her name is Maureen. She's 80 years old and living in a small house in the beautiful mountains of North Georgia.

Her childhood dream had been to care for abused and neglected animals through an animal rescue. Maureen depends on Medicare for her health insurance and social security for her income, like so many millions of Americans at her stage in life Maureen lives check to check. She's taken care of herself over the years and describes herself as very healthy.

Unfortunately, as she aged Maureen developed blood clots in her leg and lungs that threaten her life. To save her health Maureen was prescribed anti-blood clotting medication. She will need to be on this treatment for the rest of her life, and Maureen is required to pay $400 every 3 months just in cost sharing for this treatment.

And at that price Maureen simply cannot make ends meet. So Maureen has given up all of the non-essential expenses in her life. She's given up almost all the driving to save on gas and maintenance costs. She can't afford to go to a dentist, but that still isn't enough. And so Maureen tells us that she's made the incredibly heart-wrenching decision to cut back on food.

Maureen is limiting herself to eating one meal a day. And when hunger sets in she drinks water because it fills her up. These are the impossible tradeoffs people are making as a result of our broken system. An 80-year-old woman has made the decision to give up food to pay for prescriptions, it's unconscionable.

Maureen is a survivor, and she's resigned. But in her own words 'funding big pharma is not in my social security budget and here I am.' Congress created the problem of out of control drug prices and time for action is long past due. Thank you for holding the hearing. Thank you again for inviting Families USA to be here. To the Committee Members I say let's get this done, let's send the Lower Drug Prices Now Act to the President. It would be a political victory for the entire Congress and change the lives of Maureen and millions of others.

[The prepared statement of Mr. Isasi follows:]

PREPARED STATEMENT OF FREDERICK ISASI

Testimony of Frederick Isasi, JD, MPH
Executive Director
Families USA

Before the House Education and Labor Committee
Subcommittee on Health, Education, Labor, and Pensions

Lower Drug Costs Now: Expanding Access to Affordable Health Care

May 5, 2021

Families USA
1225 New York Avenue, NW
Suite 800
Washington, DC 20005

Chairman DeSaulnier, Ranking Member Allen, and members of the House Education and Labor Committee, Subcommittee on Health, Education, Labor, and Pensions: Thank you for the opportunity to speak with you today. I am Frederick Isasi, the Executive Director of Families USA. For more than 40 years, we have served as one of the leading national voices for health care consumers both in Washington, D.C. and on the state level. Our mission is to allow every individual to live to their greatest potential by ensuring the best health and health care are equally accessible and affordable to all.

I am delighted to be speaking to this subcommittee at this pivotal time. Some of you may remember that I testified before this subcommittee two years ago. While so much in our world over the last two years has changed dramatically – including the biggest health and economic crisis our country has weathered in a generation – when it comes to drug prices, the story is frustratingly much the same.

After decades of egregious price increases and staggeringly high-launch prices, it is time to solve the problem and reduce the burden of prescription drug costs on America's families. Poll after poll shows that this is a pressing concern for the vast majority of people in this country, no matter their political persuasion. Nearly 9 out of 10 voters want Congress to act to address high drug prices and *to do it this year*.[1] Congress created this problem by giving pharmaceutical companies the monopolies that they abuse; and ultimate only Congress can solve this problem. And every year that we allow this to continue not only do people face illness, death and financial ruin; but we incentivize the tricks of drug companies and their lawyers, instead of rewarding the innovative, life-saving drugs we all want and need.

Ninety-five percent of voters, across the aisle, overwhelmingly support giving Medicare the power to negotiate for lower prices.[2] Now is the time to build on the unprecedented progress made in the 116th Congress to finally realize the will of voters. On behalf of the millions of families struggling every day to afford prescription drugs, thank you and the entire Committee, including Chairman Scott and Ranking Member Foxx, for your leadership in this effort.

The Impact of High Drug Prices on Families

While high drug prices are a source of seemingly constant policy debate in Washington, D.C., for millions of America's families, they are a painful and burdensome reality that often impacts their basic necessities of life. For example, consumers facing increased drug costs report cutting-back on key areas of their budget, such as buying food.[3] And for some, the choice is even more dire. Incredibly, nearly three in ten adults – approximately 80 million people – in our country have not taken required medicine due to its costs.[4] And, approximately one in five forgo essential medications altogether because they can't afford to fill their prescription.

While people who need high-priced drugs often face the most significant financial pain from high and rising prices, the impact of the skyrocketing cost of drugs is spread across all consumers. In fact, almost 22 percent of a privately-insured health care consumer's

monthly premium goes to prescription drugs.[5] And overall, people in America pay exponentially more. A recent RAND report found that drug prices in the United States are over 250% higher than prices in 32 other countries. For brand-name drugs, the figure rises to 344%. [6]

Please allow me to share the story of just one of the millions of consumers struggling under the burden of high drug costs – a woman named Maureen, who is 80 years old and living in a small house in the North Georgia Mountains:

> *Maureen depends on Medicare for her health insurance and social security for income – living check to check - and describes herself as extremely healthy with the exception of blood clots in her left leg and lungs.*
>
> *She was prescribed an anticoagulant treatment, and told she would need to take the medication for the rest of her life. She pays $400 every three months, as prescribed. But at that price, Maureen simply cannot make ends meet and live out her retirement dream of focusing on animal rescue. So, she has decided to "give up food." She eats one meal a day and drinks tons of water because it fills her up, and she has also given up the dentist and non-essential driving to save on gas and repair costs.*
>
> *There are currently no generic competitors for this class of drugs and despite brand competition, prices have been going up over the past 5 years for these life-saving drugs.[1] Maureen is resigned "you either pay it or you take your marbles and go home." "Funding Big Pharma was not in my Social Security budget plan, yet here I am. Drug prices are life-changing, and not in a good way."*

These are the impossible trade-offs people are making as a result of our broken system. An 80-year old woman *gave up food* to pay for her prescription. It is unconscionable that she should feel compelled to choose trading food for medicine.

Exploitative pricing is more than academic for those who rely on lifesaving drugs. Ten years ago, Naloxone, a life-saving drug used to treat opioid overdoses, cost just one dollar. In the midst of the opioid crisis, the price rose to $150 (for a two doses), and the auto-injectable version to a stunning $4,500.[7] Just this year, the Senate Finance Committee report on Insulin revealed a surge in prices for this century-old drug, unrelated to any new clinical benefit. They found that, rather than competing, manufacturers aggressively increased prices "in lock step", invested only a small fraction in research and development, and pocketed the additional revenues.[8] Even dexamethasone — a key therapeutic now considered a standard of care for COVID-19 and hailed for being inexpensive and accessible — increased in price by 137% in recent months, from $0.59 to $1.39 per unit, all while it sits on the Food and Drug Administration (FDA) shortage list.[9]

Debunking the Innovation Canard

At the foundation, Congress created drug exclusivity and patent laws to incentivize the real, life-saving innovations that we all want and need. There is no question that Congress got it wrong; and in the end, only Congress can truly fix the problem.

Despite pharmaceutical industry claims that high prices are fueled by the risk and cost of drug research and development (R&D), recent evidence suggests these costs make up a small share of their spending. In fact, large, brand-name drug manufacturers would still be the most profitable industry sector— while sustaining current levels of research investments — *even if they were to lower their sales by $1 trillion.*[10] Meanwhile, taxpayer-funded research contributed to every single new drug developed and approved from 2010 to 2019, totaling more than $230 billion.[11]

For decades, drug makers have systematically abused patent and market exclusivity rules to quell product competition.[12] For example, AbbVie has nearly 250 patent applications around a single product – Humira – with the aim of extending the company's monopoly and delaying competition for 39 years at an estimated cost of $14.4 billion to American taxpayers[13] And AbbVie is not alone in these abusive practices. The makers of the top 12 best-selling drugs in the United States have filed, on average, 125 patents per drug, resulting in an average 38 years of blocked competition, far in excess of the exclusivity envisioned under Federal law.[14] Instead of investing in real innovation, drug makers would rather make outsized profits on minor tweaks to existing drugs, which is why *more than three quarters of new patents are for existing drugs.*[15]

When patents on blockbuster drugs do finally expire, brand name manufacturers have turned toward increased prices on their remaining products to maintain and expand high revenues.[16] According to a 2017 study, revenues generated by new drugs failed to make up for loss in revenues due to expiration of patents. Increases in invoice prices for current drugs under exclusivity, however, generated $187 billion in revenues.[17] Were it not for these price increases, revenues for name brand pharmaceutical companies would have been flat over the last decade, and overall spending on drugs would have fallen due to increased utilization of generic drugs.[18]

And, even when drug manufacturers do allocate a small percentage of their revenue toward *bona fide* innovations, all too often they focus their resources on drugs that don't address the most urgent needs of families and instead focus on niche drugs that yield the greatest profit.[19] For example, experts agree that across the world there is an urgent need for new antibiotics to combat increasing drug resistance, but major pharmaceutical corporations continue to step back from that life-saving research.[20]

It is clear that the current drug exclusivity and patent regime is achieving the opposite of its goals. Instead of incentivizing true, life-saving innovation, it is incentivizing high-powered lawyers to leverage loopholes in the law to make some of the largest profits in the world. And as a result, every year that Congress continues to fail to act, is another year that drug companies play their tricks and all of us *do not* get the benefit of the real, life-saving innovations that could be achieved with improved financial incentives.

COVID-19: A Novel Coronavirus with the Same Old Story

Certainly COVID-19 vaccines are extraordinary public health advances. The speed at which they have become available could not have been anticipated, and as a result, millions upon millions of lives will be saved. But let's be clear, we hear a lot about the industry taking on financial risk to produce these innovations and rarely hear about the significant financial investment made by the average person. Across all COVID-19 vaccine development, taxpayer funded government and charitable investments account for almost 43% of dollars invested, including 100% of the Moderna vaccine. Return on investment is coming swiftly to shareholders. Wall Street forecasts $38.5 billion in sales for the top five COVID therapeutics in 2021. Pfizer is projecting $15 billion in sales this year and their CEO alone collected $21 million in 2020.[21] But, families in America, almost half of whom don't own stock, are tightening their belts and waiting for their return on investment. At the same time, drug makers increased prices on a record-setting 900+ drugs before the end of January 2021.[22]

Anticipating that they may have to pay twice (for the fundamental research and development and then again at the pharmacy), Americans have expressed overwhelming support across party lines for legislation that would cap the price on any COVID-19 vaccine or treatment developed with federal funding. In fact, the pandemic has elevated the urgency of addressing high prescription drug costs, especially among Black, Latinx, and younger voters. [23]

Current Medicare Drug Payment Policy Represents Total Market Failure

In his first address to a joint session of Congress last week, President Biden reiterated his call to action on drug pricing and specifically pointed to Medicare-based solutions:

> "*…let's lower prescription drug costs. We all know how outrageously expensive they are. In fact, we pay the highest prescription drug prices in the world right here in America – nearly three times as much as other countries. We can change that. Let's do what we've always talked about. Let's give Medicare the power to save hundreds of billions of dollars by negotiating lower prices for prescription drugs. That won't just help people on Medicare – it will lower prescription drug costs for everyone.*"[24]

Critics of a move to allow Medicare to negotiate on prices claim that these bills will "would end the current market-based system."[25] To suggest that the current way in which brand name drugs are purchased by Medicare as "market-based" is utterly absurd. In truth, Medicare payment for brand name drugs is as far from a competitive marketplace as can be imagined. First, Congress has granted government-sanctioned monopolies on brand name drugs through patent and market exclusivity laws. Second, Congress tied Medicare's hands by barring it from negotiating on prices for these drugs. Finally, Congress legally bars the government from saying no to drugs at exorbitant prices. *Let us be very clear: this is not a competitive market. It is a hostage situation.*

State Remedies are Limited without Action by Congress

Many states are doing everything in their power to address the drug affordability crisis for their consumers but they need the federal government to take action if they are to have the ability to fully address high and rising drug prices. During the 2021 legislative session, state legislators in 44 states have filed 275 bills to control drug costs.[26] Fourteen states have introduced legislation to create Prescription Drug Price Review Boards, which increase access to certain high-cost drugs by determining an affordable rate.[27] Other states have introduced legislation to use international reference pricing approaches or to address egregious price increases on drugs that show no evidence of additional clinical benefit.[28] These state efforts are challenged by lawyers from the pharmaceutical industry based on the theory that their ability to set outrageous prices comes from the U.S. Congress and federal law. And, without action from the federal government, state legislation can only do so much. Congress created the rules that drug manufacturers have so blatantly abused, and it alone has the power to truly solve the problem.

Legislation under Consideration

One option Congress is currently considering to reduce drug costs is H.R. 3, *the Elijah E. Cummings Lower Drug Costs Now Act,* which represents a critical and clearly necessary step in addressing the rapidly-growing crisis around prescription drug costs.

The *Elijah E. Cummings Lower Drug Costs Now Act* is the kind of legislation consumers are demanding—it requires government to take action so that they can afford their medicines without bankrupting themselves in the process. And it does this without risking access to lifesaving medicines through a restrictive formulary. Specifically, the *Elijah E. Cummings Lower Drug Costs Now Act*:

- Authorizes and mandates that the Secretary negotiate directly with drug manufacturers on insulin and at least 50 other drugs that lack competition with the greatest costs to Medicare and the U.S. health system.
- Establishes a maximum negotiated price of no more than 1.2 times the average price offered in six other countries (Australia, Canada, France, Germany, Japan, and the United Kingdom).
- Requires manufacturers to make the negotiated price available to other purchasers.
- Provides a strong incentive for manufacturers to negotiate in good faith and to provide the negotiated price to Medicare and other purchasers through the use of an escalating excise tax and civil monetary penalties.
- Limits manufacturers' ability to hike the price of drugs year after year by imposing inflation rebates in Medicare Parts B and D.
- Caps out-of-pocket spending for seniors in Part D at $2000.

When enacted, the *Elijah E. Cummings Lower Drug Costs Now Act*, will significantly improve the affordability of prescription drugs for consumers and produce substantial savings in the Medicare Program. A Congressional Budget Office and Join Committee

on Taxation analysis (of HR3 introduced in the 116 Congress) found that the price negotiation provision would lower spending by $456 billion.

These savings can then be reinvested in ways that promise to improve health and health care for all consumers. Families USA supports using these savings to improve Medicare benefits, such as providing much-needed Medicare dental coverage and improved support for low-income Medicare beneficiaries, as well as critical investments in affordable health coverage for people who get their insurance through Medicaid and the marketplaces.

While Families USA strongly supports the passage of the *Elijah E. Cummings Lower Drug Costs Now Act*, we recommend several critical improvements to strengthen the bill to ensure that it fully delivers on its promise to make prescription drugs affordable. These improvements include:

- **Expanding the selection of drugs subject to negotiation:** The government should be authorized to expand, over time and with experience, the minimum number of drugs for which the Secretary must negotiate a fair price annually. There also should be stronger criteria in place to ensure that it is the price for costliest drugs that are negotiated. I do want to acknowledge that this latest version of HR 3 has been improved in this area, as the previous version of the bill included 25 drugs eligible for negotiation instead of 50. Additionally, the definition of a negotiation-eligible drug should be expanded to include drugs that face competition from less than three generics, as it is at this level of competition that prices are significantly reduced.[29] The Secretary should also have the discretion to select additional drugs for negotiation if the manufacturer is engaging in particularly abusive pricing practices.

- **Ensuring all consumers and purchasers are protected by price spikes:** The vast majority of American families below retirement age receive their health coverage through employer-sponsored insurance (ESI), others through the health insurance marketplaces, and others through Medicaid. Some of this coverage falls squarely in the jurisdiction of this Committee and this Committee must act to improve that coverage. There should be strong incentives and/or penalties in place to ensure that manufacturers cannot raise prices above the rate of inflation for non-Medicare purchasers as well. This is particularly critical for drugs which have a relatively low exposure to Medicare – such as pediatric drugs.

- **Protecting uninsured consumers:** Though under this bill manufacturers would be required to make the negotiated price available to other health plans, this leaves uninsured consumers subject to high prices. As the consumers most vulnerable to high and rising prices, Congress should ensure that uninsured consumers can purchase drugs at no more than the prices negotiated for Medicare.

The American People – Across the Political Spectrum – Want Action NOW

In the 2020 Presidential and Congressional elections, the American people sent a strong signal to Capitol Hill that they want real solutions to make health care more affordable. Again in early 2021, voters expressed concern that Congress won't go far enough, especially in addressing high cost of prescription drugs (67%).[30] Fully 82% believe that the healthcare system today works more for the benefit of the drug industries and insurance companies rather than for the benefit of the average person, a sentiment that holds true across demographic groups and partisan lines. Likewise nine out of ten employers report that drug prices are among the greatest threats to affordability of health coverage for their employees.[31] Poll after poll, year after year.

Now is the time for Members of Congress to act boldly on behalf of their constituents. I ask you today, will you support this commonsense legislation to protect taxpayers and your constituents from profiteering by pharmaceutical industry and fix the broken system, or will you side with drug makers, who hope to continue to exercise unfettered and unregulated monopolies over their products?

Thank you for your time today. Let's finally solve this life and death health and economic issue. I look forward to continuing to work with this committee and your colleagues across Capitol Hill to bring real relief from high drug prices to America's families.

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Chairman DESAULNIER. Thank you. We thank all for the testimony, and again please try to stay within your allotted time. Thank you for your articulate expressions.

Now under Committee Rule 9(a) we will go to questions, witnesses under the five-minute rule. I will be recognizing Subcommittee Members in seniority order. Again, to ensure that Member's five-minute rule is adhered to staff will be keeping track of time and the timer will show a blinking light when time has expired.

Please be attentive to the time, wrap up when your time is over and remute your microphone. As Chair I now recognize myself for five minutes.

Mr. Mitchell you and I have a lot in common. In addition to the life-saving drugs that we both get, I thought my numbers were astronomical. Yours clearly are really even more so, and as you, I'm grateful for them, and grateful for the investment that created them and keep me alive.

One of the things that I struggle with both as a client, a payer, and as a taxpayer is what's the best formula? What's the cost benefit? How do we attract private sector funding, but also recognize and continue to invest in taxpayer funding so that we get these life-saving drugs.

In your view, I have two questions, you also were a small business person as was I. I always think it's funny when I'm associated with radical socialism by some of my friends. I was a former republican small business owner who owned a restaurant named after Teddy Roosevelt. My kids would be shocked to hear me described as a radical socialist.

So in this bill, in H.R. 3 Mr. Mitchell, tell me how that would help clients like you, and I reduce the cost of our life-savings drugs, but not inhibit these investments? And I am of the mind that I'm afraid that American payers and taxpayers are subsidizing for instance, in my drug, the $500 I pay goes to subsidize the lower price for Australians with the same diagnosis.

And then second, as a small business, former small business person, describe some of the pressures that you are under to provide health insurance for your employees when the costs are so exorbitant.

Mr. MITCHELL. Mr. Chairman, first of all, I'm sorry that we share the experience of having cancer. I'm glad, very glad, that both of us have drugs that are working right now. I believe it's important for us to have an approach to setting drug prices where we incentivize the development of the most valuable new drugs.

Drugs that carry high value should get a high price. A drug that cures my cancer should command a very high price, because there isn't one that will do that, or a drug that treats Alzheimer's effectively. So, I want a system that incentivizes investment in drugs that will make a difference and meet unmet needs.

And we need to make sure that we're sending enough money to NIH to focus on drugs that are public health priorities, not necessarily private profit priorities. I ran a business for almost 30 years. It is very difficult when the cost of health care goes up to absorb it if you run on tight margins, and what you wind up doing is either you pay people less money because you have to send it to pay for the more expensive drugs, or you shift costs onto them.

And in fact the ERISA Industry Committee issues a statement yesterday saying it wasn't very fond of the republican alternative,

H.R. 19 because it includes a number of policies that just shift costs to employers.

So we have to be very careful what we do as we try to tackle this problem.

Chairman DESAULNIER. Thank you, Mr. Mitchell. Dr. Socal, let's talk a little bit about where the money is going to. So, the United States as I understand it, is the only country along with New Zealand that allows television advertising for prescription drugs.

Could you talk a little bit about the amount of money that the pharmaceutical industry spends on marketing and sales as opposed to what they spend on research and development? And do other countries have the same formula?

Dr. SOCAL. Thank you for this question. Yes. 9 out of the 10 biggest pharmaceutical manufacturers are spending more on marketing and advertisement than on research and development for drugs. And, of course, you use the drugs that have the highest prices that can invest more in advertisement. And that is only allowed in the United States and New Zealand we are the only exceptions in the global market that allow manufacturers to advertise directly to consumers.

Everybody else believes that physicians are the ones who know when a drug works, and physicians can be trusted to prescribe these drugs to consumers. We are really an exception on that side.

Chairman DESAULNIER. Dr. Socal are you familiar with any research that attempts to quantify what the right formula is? So, what's a reasonable rate of return for private sector investors, and how do we identify the base level of research or more that taxpayers pay for that come out of the NIH?

Dr. SOCAL. What we really want is real innovation, and not innovation about 'me too' drugs. Drugs that are just copies of everything else that is already available.

Chairman DESAULNIER. It would be really helpful to get those kind of numbers so we could have a more rational, constructive conversation I think across the aisle. Because I do think we all want the same thing. We want to make sure Americans get this innovation, but we want to make sure there's a reasonable rate of return to attract those investments, not an exorbitant rate of return.

Dr. SOCAL. Absolutely.

Chairman DESAULNIER. With that I will end my questioning and turn it over to the Ranking Member, my good friend Mr. Allen.

Mr. ALLEN. Thank you Mr. Chairman. For the benefit of our Committee Members I'm going to defer to the end if I may, and we can go to our next most senior Member if that's OK.

Chairman DESAULNIER. That's fine.

Ms. FOXX. Mr. Chairman, this is Congresswoman Foxx. I believe I was to be recognized next.

Chairman DESAULNIER. OK. I didn't have that information. I would be delighted to recognize you, Virginia.

Ms. FOXX. Thank you Mr. Chairman. You know I feel very positive about you and I'm glad you're there. As I tell all our witnesses for being there today. Dr. Holtz-Eakin in addition to reporting foreign price controls and setting prices for some drugs and Medicare,

H.R. 3 gives employer sponsored plans the "choice" to participate in this government price-setting system.

I put choice in quotation marks because employers are not presented with a choice. Not only does H.R. 3 force employers into the democrat drug pricing scheme, but it also punishes those who wish to opt out and negotiate prices on their own.

Do you think H.R. 3 provides fair choices to employers and the plan participants? What are the consequences for employers who choose to opt out?

Mr. HOLTZ-EAKIN. Well thank you for the question Dr. Foxx. I don't think H.R. 3 represents genuine negotiation either between the secretary and manufacturers, or with ERISA employers having the opportunity to negotiate on their own. So this is really a very sweeping proposed legislation that affects every third-party payer in the United States, and sweeps them into a price fixing regime that I think will have really negative impacts on the U.S. and on its citizens.

So I just want to emphasize some things that have been said that I really think don't get appreciated. No. 1, the idea that we should concentrate on a reasonable rate of return is a sensible idea, but it has to be risk adjusted. One in a thousand pharmaceutical formulas gets to a trial. Of those, 8 percent get approved by the FDA.

This is as risky a business as you can imagine, and after the fact some returns look high for that reason, and that reason alone. So it has to take into account those risks. The second thing is that if we're going to have the NIH pick the drugs, which ones are they going to do? They have to pick from all those too. At least the private sector is losing their own money when they pick a drug that doesn't work out.

And the idea of 'me too' drugs being a bad idea, that's what competition, that's how it's generated. When Sovaldi came on the market as the only cure for Hep C it immediately was followed by two other 'me too' cures for Hep C which drove the price down by 50 percent.

So the only solution for high pries is better competition, and the idea that somehow the NIH is going to generate competition whether eliminating 'me too' drugs as the solution is really mistaken.

Ms. FOXX. You mentioned in your opening statement that employers are now able to negotiate price reductions without the government interfering in the commercial market. I think we understand it, but what would you say about how these negotiations benefit employees?

Mr. HOLTZ-EAKIN. Well, if you're negotiating with a drug company and you have the capacity to move large amounts of their products at a preferable price you get a better deal. And that's the kind of incentives that you want in a system. Those are the incentives that were underneath the original design of Medicare Part D, and as I noted in my remarks it will be a good idea to sharpen those incentives even further at this point in time that have eroded somewhat.

That's the route to getting prices down, provide competition and strong negotiations and allow increased supply wherever possible.

Ms. FOXX. Great. I have two more questions and the Chairman has asked us to be within our time. Dr. Holtz-Eakin employer sponsors of group health plans are required by law to administer their plans in the best interest of the employees. However, H.R. 3 could create conditions that place employers in difficult conditions and expose them to unnecessary legal risks.

Can you explain briefly why this could very well happen, and what would be the consequence to employers?

Mr. HOLTZ-EAKIN. Sure. The array of provisions that lead to the maximum fair price "negotiated by the secretary," would be available to those employers. They could also try to go out and negotiate on their own, but if they can't guarantee that they're going to get a lower price, it might not be in their fiduciary interest to do so. They would have to take the arbitrarily set government price.

Ms. FOXX. Fine. One more question. Health plans use a formula, a list of covered drugs selected by the plan to help manage costs, especially when it comes to specialty drugs. How will H.R. 3 affect the drugs included in the formulary? Will employees and their families still have access to new and innovative treatments?

Mr. HOLTZ-EAKIN. My deepest fear is that we will in fact inhibit innovation in the United States. It has happened in other countries. I mean Germany used to be the center of medical science on the globe. It's not a God-given right. They adopted bad policies and the United States is now that place.

We've got bad policies. We can lose that innovative sector and we cannot have the next generation of therapies.

Ms. FOXX. Thank you very much and Mr. Chairman I yield back within time.

Chairman DESAULNIER. Thank you, Dr. Foxx, you get a gold star. Next, I'm going to recognize the gentleman from Connecticut, Mr. Courtney.

Mr. COURTNEY. Thank you, Mr. Chairman and thank you to all the witnesses for being here today. Mr. Mitchell, I wanted to again thank you also for correcting the record, or just adjusting the record regarding the discovery of the COVID vaccine.

Actually, just 3 weeks ago the U.S. Patent Office awarded the NIH a patent on the spike protein technology which Pfizer and Moderna used to basically come up with their iterations of a vaccine. Again, the taxpayer took a lot of the risk out of the research that went in, and again as you point out that actually proceeded the outbreak of the virus.

I think almost every other Member on this Subcommittee with all the COVID relief bills that we voted on going back to the original or even pre-CARES Act, billions were approved on a bipartisan basis, which for all of us I think should you know feel good about in terms of you know trying to accelerate and create the warp speed mechanism that again was a great success.

And the companies who were involved in that kudos to them, but you know it is clear that the taxpayer and NIH were instrumental, even to the point of having legal ownership. Mr. Chairman, I would ask that the patent notice be admitted to the record. Again, just to confirm the government and the taxpayer's role in terms of getting us to the vaccine.

Chairman DESAULNIER. Without objection.

Mr. COURTNEY. Thank you. And Ms. Socal you know I speak a lot to the Connecticut Insurance Department. We have a regulated insurance market where rates are approved in both the individual market and the business market. And last year the insurance commissioner reported that the share of premiums that employers pay for prescription drugs is now at about 23 percent of premium dollar.

If you go back really even just a few years ago that was at 15 percent. So I mean do you see that trend in employer-sponsored plans in terms of how much prescription drugs are driving the increase in premium?

Dr. SOCAL. Yes, we see that a lot. Not only increases in premiums, but really increases in the overall spending that patients make in their out of pocket costs as well.

Mr. COURTNEY. So, again, there are obviously a lot of other health care costs that go into the premium dollar, you know, whether it's you know hospital reimbursement, physician reimbursement.

So, again, it's really disproportionate in terms of again, the trajectory that we're seeing right now which really is why I think this bill which sometimes when you talk about prescription drug costs, it's all sort of Medicare. This bill is not just about Medicare, it's also about providing relief for employer-sponsored plans.

Dr. SOCAL. Absolutely. And a lot of what's driving these costs is these very high, very expensive drugs that really don't offer any additional value as compared to all the alternatives that are in the market, but they are just increasing their prices, and that's really unbeknown to patients, and often to physicians as well.

Mr. COURTNEY. So again, as someone who was also a small employer at one time, you know I wanted to sort of go back to this point about what the employers sort of fiduciary responsible, what his options are, again this bill does not mandate that an employer purchased a health care plan that has the government negotiated price right?

I mean they still have an option to use market or PBMs or whatever to get their prescription drug coverage is that right?

Dr. SOCAL. They do have an option, and that option would bring really important transparency to this market because even if a self-insured employer is hiring a PBM to negotiate on their behalf, often times the employer does not even know exactly how much they're paying for drugs.

There's an important lack of transparency there, and this bill would bring a lot of transparency back for employers to know what's best for them.

Mr. COURTNEY. And transparency is really what is essential for market economics, isn't that correct?

Dr. SOCAL. It is crucial.

Mr. COURTNEY. I mean that's the irony of some of the rhetoric here. This bill actually provides a healthier marketplace, not a more restricted marketplace isn't that correct?

Dr. SOCAL. It is. And part of the lack of transparency is having so many intermediaries in our negotiations, and having a transparent benchmark will help employers to be able to even monitor the work of these intermediaries more effectively.

Mr. COURTNEY. Well thank you for your testimony, and Mr. Chairman in the spirit of moving along I yield back the balance of my time.

Chairman DESAULNIER. You get a gold star as well with Dr. Foxx Mr. Courtney. And with that the Chair will recognize Mr. Wilson from South Carolina for five minutes.

Mr. WILSON. Thank you Mr. Chairman. And indeed Joe Courtney deserves a gold star. Additionally, I appreciate Ranking Member Rick Allen proceeding as we're doing today. And also I want to ask a question to Dr. Holtz-Eakin. The U.S. Chamber of Commerce has estimated sadly that in my home State of South Carolina 4,401 jobs would be destroyed through the H.R. 3.

The consequence of a big government power grab leading to delay and denial of medical care with lack of access. On top of that the pensions and mutual funds hold a significant amount of my own pharmaceutical shares on the S&P Index. How do you see H.R. 3 affecting pension plans and retirement accounts in the short and long run?

Mr. HOLTZ-EAKIN. Well certainly in the short run this would be a big negative for the market value of those holdings and that would hurt the funding of those pension plans. I think that's very straightforward. Over the longer term fully implemented, this is a real negative for the entire sector for the small startups that develop drugs and often sell them and always plan to sell them to larger pharmaceutical companies.

And that value that has always been a part of the system would dry up and disappear. So over the long-term this would be negative as well. So I don't think there's any question about the impact of this.

And you know I'm very sympathetic to the notion that especially for specialty drugs right now, and a lot of those are oncology drugs, that prices are very high, and that there's some severe financial distress for Mr. Mitchell, and Chairman, and people like them who are kept alive by those drugs.

They're an enormously expensive, but valuable item. The irony is that although that's a very difficult situation, H.R. 3 would do nothing to make it better. It would only make it worse. There would be no innovation to replace them with something that is a cure, or anything that is accomplished and lowers the price, and I think that's the real concern.

Mr. WILSON. Well thank you for your analysis and another question for you Dr. Holtz-Eakin, I'm grateful that Nephron Pharmaceuticals, a well-known business located in Lexington County, South Carolina. The President, CEO and owner, Lou Kennedy, who's a real superstar in our community recently announced a 240,000 square foot expansion to include 110,000 square foot vaccine production, chemotherapy and antibiotic wing.

This expansion is estimated to create 380 new jobs in our community. What kind of job reduction could occur within the pharmaceutical industry if H.R. 3 were enacted?

Mr. HOLTZ-EAKIN. We would see fewer innovations. The CEO is on record as saying there would be fewer innovated drugs. The Council on Advisors estimated a larger number. Directionally everyone knows the answer, the only question is how big is it, and

that means that you don't build those factories, and you don't hire those individuals.

Mr. WILSON. And hey, I would invite everybody to come visit, Nephron Pharmaceuticals is really very close to my house, and it's so exciting to see the newer cars in the parking lot. But it also Dr. Holtz-Eakin, through operation Warp Speed with the leadership of former Vice President Mike Pence, we've seen the American ingenuity at its finest.

As Ranking Member Rick Allen has accurately reviewed, with the incredible success of development. The United States should maintain that capability to avoid reliance on international competitors like China, which sadly disregard lawful practices. With China aggressively advancing their research and development capacities, and you see H.R. 3 impacting our capabilities and international competition.

Mr. HOLTZ-EAKIN. I think it's an unambiguous negative for innovation. I've said that several times now. To the extent that the Chinese are piling money into innovation and trying to compete on that front, it's a handicap. And I want to take this opportunity to thank everyone on this Committee, and in Congress on both sides of the aisle for Operation Warp Speed for the funding that you provided.

That was an extraordinary accomplishment. The MRNA technology had been around for three decades. There's been a lot of private risk capital put in there for things that did not pay off, and in the end by financing trials that overlapped and guaranteeing markets for the vaccine that was being manufactured although not approved, you did something remarkable in conjunction with the private sector, and I think it was a great accomplishment.

Mr. WILSON. Thank you very much for your insight and I yield back.

Chairman DESAULNIER. Thank you, Mr. Wilson. Everybody is getting a gold star so far. Well now I will go to Mr. Norcross from New Jersey and I'm certain he will get a gold star.

Mr. NORCROSS. Absolutely. Thank you, Chairman, for holding this hearing. Dr. Holtz-Eakin help me understand something just backing up. You suggested that Germany used to be the most innovative place in the world, and that they passed some regulations, and they moved to the U.S. as most innovative. Does that encapsulate what you were trying to get across?

Mr. HOLTZ-EAKIN. That's the short version of the long argument yes.

Mr. NORCROSS. So, does that mean now that the U.S. has that, that Germany you're not going to give any of the innovative drugs to them because you're no longer there?

Mr. HOLTZ-EAKIN. No. I'm just saying that we have had a very effective system at nurturing innovation in pharmaceuticals.

Mr. NORCROSS. Absolutely. But you suggested that Germany did also. They've lost it now because they passed some laws. So, the first question is so what did they lose? That innovation you won't send them any of the new drugs?

Mr. HOLTZ-EAKIN. It's not about where you send them, it's about what gets developed where.

Mr. NORCROSS. Wait, well, hold it. If it's developed does that mean you're not going to share it with another country? You're not going to sell it there?

Mr. HOLTZ-EAKIN. I never said anything about where it was sold.

Mr. NORCROSS. I'm asking.

Mr. HOLTZ-EAKIN. It will be sold.

Mr. NORCROSS. I'm asking because they're not innovative, but they moved out and somehow they're not going to get those drugs. But I think you answered the question correctly, they will get those drugs. They might not get them the first day, but they're going to get them, thank you I appreciate your answer.

Mr. HOLTZ-EAKIN. Just for the record and the testimony, about 40 percent get them.

Mr. NORCROSS. I'll claim my time.

Chairman DESAULNIER. It's the Member's time please. Go ahead, Mr. Norcross.

Mr. NORCROSS. Dr. Socal, I used to be part of a local union and we took care of our health and welfare funds trying to always save costs and be innovative. And then came along these pharmacy benefit managers, it sounded like the best thing since sliced bread.

They promised us the world. And they did for a while. Now what we found out is that the negotiations that they're able to make are not transparent. We're not getting the benefit that we used to because that's not disclosed. Can you talk about the PBMs, what works well and what doesn't?

Dr. SOCAL. Sure. I fully agree with you at the beginning they were needed, especially with so many different new drugs entering the market. But the problem is that PBMs not only negotiate, but they also make a profit off the drugs that they do negotiate, so they make a cut.

And it is in their best interest oftentimes when they have two different options, one is lower costs and the other one is higher cost. It is in their best interest oftentimes to have the higher cost drug in the formulary, so that they can make a higher cut of the price of that drug.

And that's one of the reasons why the prices set by manufacturers are increasing so much today where the prices that end up being negotiated have seemed to be often times just stable, or not growing as quickly. And for patients this is very, very harmful, because there is cost-sharing that is paid over those prices set by the manufacturers and the PBMs are simply making a cut while everybody is negotiating a lower price.

Mr. NORCROSS. So just to understand, the PBMs came into existence because they could gather a larger group of customers, put them together and give you a better chance against the big pharma, so that worked.

If we were to put transparency in there, would that go a long way to fixing that problem that you just suggested?

Dr. SOCAL. It would fix part of the problem, especially for these 'me too' drugs, but another part of the problem is drugs that do not have any competition, they're not 'me too'. They are very important, and I just gave an example today in my testimony.

And for these drugs the PBM cannot say no, they have nothing to compete this drug against and try to lower this drug price. And

for these situations the PBM negotiation model is just not successful.

Mr. NORCROSS. Thank you and Mr. Chairman I yield back with 45 seconds left thanks.

Chairman DESAULNIER. Oh those precious 45 seconds of your wisdom. We will now go to Mr. Walberg, the distinguished gentleman from Michigan.

Mr. WALBERG. You're a really kind Chairman and I say thank you for that. Thanks for the hearing. There's no question that healthcare costs are at the top of the minds for many Americans, myself included, and the cost of prescription drugs are a concern for workers and families.

Sadly, instead of working on bipartisan solutions we all agree on, the bill we're discussing today seeks to impose radical price setting policies that run the risk of decimating biopharmaceutical research and jobs.

In Michigan, a State that is proudly producing the COVID–19 vaccine 86,000 jobs are supported by the biopharmaceutical sector. Moreover, the bill will reduce the necessary investment in research and development and access to breakthrough cures for difficult and rare diseases like Alzheimer's, childhood cancers, sickle cell anemia just to name a few.

We all have met families, or know someone who have felt the devastation when a loved one has been diagnosed with a disease with no cure. So it's disappointing that we are here again, holding a hearing on this socialized agenda, drug pricing scheme that would further dash the hopes of finding breakthrough cures, and I believe saving many, many lives.

But we ought to talk about it, so here we are. And Dr. Holtz-Eakin thank you for being here with the rest of your panel. You note in your testimony that it takes on average 2.9 billion dollars and 15 years to bring a successful drug to market. And many drugs are never approved.

How would putting the HHS Secretary, and Washington bureaucrats in charge of price setting and political risk to the already risky process of developing and delivering drug treatments to Americans?

Mr. HOLTZ-EAKIN. It would add an enormous amount of risk. You'd be exposed to the starting point, which is the average indexed international market price, that's something you don't know and when it's in the control of bureaucrats outside of the United States, you would then have the secretary having the unilateral power to declare whether negotiations were in good faith or not, run the risk of getting the drug essentially taxes out of the domestic market entirely, having no revenue.

Those are just recipes for increased risk and less capital flowing to that industry. Everyone always thinks the drug companies somehow are going to get in the fetal position and stop developing drugs. No. That's what they do.

But then no one will give them the money, and that will be the reality.

Mr. WALBERG. That just makes common sense, which maybe is not all that common. Dr. Holtz-Eakin we know that China is striving for a bigger footprint in biotechnology as to be assumed. I'm

concerned that the policies put forward in H.R. 3 would make the United States more reliant on other countries like China for research and development.

Can you speak to what repercussions, greater reliance on China for that medical breakthroughs would have on the United States?

Mr. HOLTZ-EAKIN. I think there are two big concerns. No. 1 has been the well-noted issue of security of supply chains for those drugs that are already in production, and it's a sensible question to ask whether you want something in China or not, and if not make sure that you have something that will survive some sort of stress test as a supply chain outside of China or in the U.S.

And the second is where will you go for innovative and important therapies that are not produced in the United States, but which our citizens would like to have? And that's a situation that I think people are increasingly finding too problematic as the Chinese continue to not play by international rules, trade on level terms with others, and thus violate their international obligations.

Mr. WALBERG. We've certainly seen that over the course of the last year haven't we? Dr. Holtz-Eakin you note in your testimony that the sweeping nature of the proposed reforms in H.R. 3 do not just apply to Medicare, but would also apply to plans operating under ERISA. If H.R. 3 becomes law, how would employer sponsored health plans be impacted?

Mr. HOLTZ-EAKIN. They end up in the same pricing regime. I don't see any way around it. I mean the starting point is the average international market price, you know that becomes the maximum, and then an ERISA employer could try to negotiate and get a lower price, but they've got their employees and their formula. The secretary has a 95 percent I will levy this and you're out of business club in their hand there's no question the secretary is going to cut a better deal.

It's just this is not a negotiation. This is using the fiat power of the government to set prices.

Mr. WALBERG. I appreciate the response, so thank you and I yield back 7 seconds.

Chairman DESAULNIER. Thank you, Mr. Walberg, I appreciate that. We will now recognize, the Chair will recognize the gentlelady from Georgia, Representative McBath the floor is yours.

Mrs. MCBATH. Thank you, Mr. Chairman. And thank you to all the witnesses for being here today. This is really great information and very insightful. As a two-time breast cancer survivor myself, and having been in you know the system, so to speak, I know all too well you know the stresses and all the heartache of the life changing diagnosis when you get it.

And then when you have it a second time it's even more challenging. I know that my treatment was exhausting, it was both physically and emotionally exhausting, and I was truly you know, blessed to be able to afford my medications. And unfortunately, we know that that's not a reality for a lot of Americans, and in particular, under all the additional stresses of COVID–19, I can't even imagine what it's been like for persons that have pre-existing conditions and have been suffering so.

And at a time when you know everyone is suffering so financially, lowering the cost of their prescription medications can really

be a very vital lifeline. Time and time again you know, I talk to my constituents all the time, and they're telling me that the cost of their prescription drugs is you know top priority for them, and how can you blame them?

It's a top priority for me. Just as of yesterday having finally, after a year, finally found a program that would reduce my costs. I also have an eye disease and I finally, finally after a year found a drug company that would actually reduce the cost of my eye drops, and I was just so grateful for that.

You know there are life-saving medications and people are having to make unthinkable decisions about whether to purchase their medications, or put food on the table or gas in their car, and the American people are just sick and tired of seeing endless prices increase and increase every year while our pharmaceutical companies see record profits.

And we just need to find a different way. Drug companies often argue that you know we've already had a system in which prices are negotiated by health plans and the pharmacy benefit managers, and therefore it's not needed for the Federal Government to negotiate directly.

However, I can clearly see that the system as it currently is designed is failing millions of patients in my district, and throughout the country. And this legislation that we're talking about today takes really bold steps toward reducing the cost of prescription drugs, and it really will save the taxpayers billions of dollars over the next decade.

So, let's be clear. It is taxpayer dollars and wages that go toward paying for these outrageous prescription drug prices. And Dr. Socal you mentioned in your testimony that you work with the business organizations who are looking to lower the cost of health care in the United States.

And I'd like to hear about what you've learned through this work. What are some of the challenges that businesses face in affording drugs, and how does this impact workers? And this is a two-part question, also how would having a price negotiated by the Federal Government help those businesses?

Dr. SOCAL. Well let me start by addressing this very important question about how employers are dealing with this problem. And employers and employees they are in the same boat, because it is very frequent that employers in their benefit design they have to charge a certain percentage of the drug cost when a patient needs a certain drug.

And charging that percentage of the drug cost is what really is detrimental to patients because the price that is available to patients when they calculate this cost, is always the highest price, and it is different than the price that is negotiated at the end of the day for the employer.

Now employers would pretty much prefer to have this revenue available to invest in retirement plans, better compensation, to keep their workforce to offer better benefits and they aren't you know capable to when they spend so much on prescription drugs.

At the same time charging a percentage of the drug costs to employees it is a way to shift the drugs cost and penalize employees when they need a very expensive drug. Having the secretary nego-

tiate these prices on behalf of everyone would allow patients to also reduce their drug costs.

The GAO report has shown that other countries for the same drugs people pay much less, and it's not that these countries have more money available to cover drugs, it's really that they have better price controls, and price negotiations in place and we can match these prices around the world if we enact certain negotiations like in H.R. 3.

Mrs. MCBATH. Well thank you so much for your answer, and thank you, Mr. Chairman. I yield back the balance of my time.

Chairman DESAULNIER. Thank you Representative, and thank you for your personal story. The Chair now will recognize the gentlelady from Tennessee Representative Harshbarger. The floor is yours.

Ms. HARSHBARGER. Yes sir. Thank you Mr. Chairman and Ranking Member Allen and the witnesses. There's no doubt we have to do something in Congress about drug pricing, and we know Americans, that's one of their No. 1 priorities, but they still want access to life saving medications, and as most of my colleagues know I've been a pharmacist for 34 years, so nobody on this call has heard more about lowering drug prices than I have over 34 years.

And I could talk to you all day if you want to know about PBMs, I'm your woman OK, because we deal with those. And I just had a roundtable the other day with independent pharmacies from the district, myself and Betty Carter, Representative from Georgia.

And did we get an earful because they are putting 2,000 independent pharmacies out per year because they won't even reimburse the price of the medication. So what I really want to touch on is I want to talk about the pharmacy benefit managers. You know these managers were created as middlemen, and they were supposed to reduce the costs of the insurers, validate eligibility, administer drug benefits, negotiate costs between pharmacies and healthcare plans.

But what has happened is they morphed into one of the most highly concentrated, least accountable profit centers in our healthcare industry. The vertical integration from the FTC should have looked at years ago that a pharmacy can buy a pharmacy benefit manager is ludicrous.

They have enormous power too over drug companies. And I use the analogy that you have the drug company as the parent, and now we have a child down here called the PBM. And now this child has grown up and it's smacking the parents around, telling them what they're going to rebate, what they're going to give them back.

So these PBMs do this. They choose what drugs are covered by insurance, they negotiate purchasing deals with drug makers, they determine the copays for the customers and what tiers they're in. They decide which pharmacies will be included in prescription drug plans, and they decide how much pharmacies are reimbursed for the drugs they sell.

It's pathetic. And big pharma, you know, the way they price these drugs, it's not even AWP which is average wholesale pricing. They made another I don't even know what it's called. It's NADCS or something, it's something made up that the way they reimburse

pharmacies, and these plant sponsors think they're transparent and they are not. They're the least transparent.

There's things called spread that they have, and they may pay a hospital pharmacy one thing, and a retail pharmacy another. And they're at their mercy. It's a take it or leave it mentality when it comes to these independent pharmacies. If they don't take it they can't stay in business. If they do take it they're going to go bankrupt eventually.

And the bottom line, they've been doing this the last 10 years, and we're to a point where these independent pharmacies are screaming at us saying help us, we're not going to survive the year. I have a PBM accountability bill out right now that I want everybody to know about. It's H.R. 1829. Everybody has an independent pharmacy in their district, and I would implore you look at that.

You know there's three big PBMs and they control 77 percent of the market. It's pathetic. So there is no transparency, and we need to fix that. But my question is for Dr. Holtz-Eakin. Do you believe the Centers for Medicaid and Medicare Services have the regulatory authority to implement some PBM reform and transparency policies already with additional legislation?

Do you think we can do that?

Mr. HOLTZ-EAKIN. Well certainly in recent years there have been efforts such as the rebate rule which was an attempt to take the list price and the net price, which is after rebates, and make sure that got passed through to the retail price out of which people would calculate their coinsurance and thus their out of pocket.

And then so one of the reasons I went through all those terms in my written is to emphasize that patterns look very different in what you use as the measure of price. So they attempt to do that. That rule in the end did not get finalized. That would have applied only however to government programs.

Congress could do something legislatively which applied to the commercial market as well if it wanted to do that, but certainly the notion that coinsurance is calculated off the list price and the net price is what everyone else is operating on, is the real source of financial distress for a lot of beneficiaries and a lot of patients.

Mrs. HARSHBARGER. Absolutely. Mr. Mitchell as you know there's a number of abusive and harmful PBM practices such as the spread pricing, the direct and indirect enumeration fees, for PBMs underpaid pharmacist and discounts to patients at the pharmacy counter.

And there's just so many practices that people need to be aware of. Since our Subcommittee has direct jurisdiction over ERISA, I'm wondering what legislative recommendations you have or would support for the Committee to take action with?

Mr. MITCHELL. Well first of all as a patient I find it very disturbing that I can't know if the preferred drug on a formulary is the best drug for me, or the least expensive drug for me, or if it's just there because the PBM got a big kickback from the drug company.

The headwaters of the problem are high drug prices set by the manufacturers, but everybody downstream is making money too, and PBMs among them. And there's a reason that biosimilars are having a hard time entering the market to compete with biologics,

that is because the drug companies negotiate rebate dwells and other arrangements to block competition working with the PBMs.

So I feel strongly we need to start with list prices, but there's work to do on this issue of transparency, and ensuring that PBMs are operating in the interests of the people they're supposed to serve.

Mrs. HARSHBARGER. Thank you Mr. Chairman. I have lots of other questions I'll submit those. I yield back.

Chairman DESAULNIER. Thank you. I really appreciate your perspective and look forward to having further discussion about your bill and other ideas. I've got a wonderful relationship with Mr. Carter. He's a co-Chair with me at the Cancer Survivor's Caucus so we'll look at these issues..

Mrs. HARSHBARGER. Fantastic thank you sir.

Chairman DESAULNIER. With that I will recognize the gentleman from Michigan Mr. Levin for five minutes.

Mr. LEVIN. Thank you, Mr. Chairman. No teacher has ever given me a gold star, so I don't know if we're going to start today or not, but.

Chairman DESAULNIER. There's always a first.

Mr. LEVIN. I'm a two-time cancer survivor like the gentlewoman from Georgia, and I'm in your club, and don't wish it on anybody else, but I also have two young adult kids with Crohn's disease and have had it for years.

So, you know this drug price issue has been very personal for my family. Mr. Isasi, you pointed out in your testimony that rising drug prices are having a direct impact on the kind of health care that patients receive. One in three Americans report skipping doses of their medication due to cost, and millions of Americans, including my constituents who tell me about it, are splitting pills, or taking other steps to stretch out their prescriptions.

So, my question to you, and I want to get through another one after this is what impact does this have on patient's health itself, and on the costs they face if they eventually do seek care after they forego their medication?

And do patients in other countries have this problem? And how do their health outcomes compare to patients in the U.S.?

Mr. ISASI. Thank you very much for that lead into the question. I think you did a beautiful job describing in your own district that so many Americans live with as you mentioned, about a third of Americans right now cannot afford their drugs, and are doing things like splitting drugs.

And as I talked about in my testimony Maureen has made the decision to cut back on food because she can't afford her drugs. So it's a very, very serious problem. When you compare what's happening in the rest of the world, this is a very uniquely American problem in comparison to the rest of the developed nations.

Most families across the developed countries do not have to worry about whether they're going to keep the roof over their head, keep a savings that they worked so hard to make, and the health of their kids and their family. And that's a uniquely American problem and it's one that needs a solution.

You know and if you think about what's happened just this year with the COVID vaccines, we are in a situation in which the entire

regime around the development of these vaccines is what H.R. 3 would put in place. The government helped to fund the vaccine development, the government negotiated the price right, and the vaccine is here for Americans.

We paid for 100 percent of the Moderna development, and we paid for almost half of all the other ones, and yet we still have record profits occurring, billions of dollars are going to Pfizer.

Mr. LEVIN. And I might point out that the companies are resisting us getting chips waivers so that these drugs can save lives around the world, and we can stop a new variant from developing and coming back.

Let me get to my second question. In your testimony you talked about also the need to ensure that all consumers are protected from price hikes, not just consumers covered by Medicare. So let me ask you to talk a little bit more about why that's important in your view, and why doesn't the protection for Medicare consumers confer protection to other consumers like those of employer sponsored health care?

Mr. ISASI. Right. Really important question. And to start with let's recognize that. What doesn't make sense is the pharmaceutical industry right now is the one industry that isn't actually by subject to the government getting in and saying is this a fair price.

Hospitals live with it. Doctors live with it. There's some reason that we've given the pharmaceutical industry in the past and that's where the abuse comes from. Second, this discussion about this radical idea that we should be internalizing the costs in Europe so that we are not substituting the drug costs for other countries. That is something that President Trump proposed and signed an executive order on in July right.

It is not a radical idea. It's something President Trump himself said he was going to do. And to your point fundamentally about 150 million Americans are currently getting their health insurance coverage not through Medicare or Medicaid right? Those folks are getting their coverage through employer-sponsored insurance.

Mr. LEVIN. Which is what we have jurisdiction over.

Mr. ISASI. And that is what this Committee works on and fundamentally we've got to solve this problem for all Americans, not just for the Medicare recipients or Medicaid.

Mr. LEVIN. All right. With that Mr. Chairman I will earn my first ever gold star. I yield.

Chairman DESAULNIER. As they say priceless. Next the Chair will recognize the gentlelady from Illinois, Mrs. Miller.

Mrs. MILLER. OK, can you hear me?

Chairman DESAULNIER. Yes I can go ahead, the floor is yours.

Mrs. MILLER. OK. OK thank you Dr. Holtz-Eakin again for your testimony, and for taking time out of your day to share your expertise with the Committee. I want to give you an opportunity to clarify your response to my colleague who asked about international access to drugs.

I was interested in your response, but unfortunately you were cutoff before you could fully answer the question you were asked, so could you answer for the Committee what kind of access countries with policies like those in H.R. 3 have to innovative medicines?

Mr. HOLTZ-EAKIN. Far less than in the United States Congresswoman. The typical comparison country in H.R. 3 has at most 60 percent of the brand name drugs that American consumers enjoy, often only a third or 40 percent, Australia, places like that. So the answer is often no, they won't have access to that drug, and indeed one of the characteristics of those countries is they denied their citizens access to the latest therapies and drugs on a regular basis.

And the United States does not do that. And we have issues that I acknowledge in some targeted situations for the financial burden of those therapies, but the advances in medical science I don't think is in dispute. The question is how we're going to effectively finance recovering the costs of developing them.

Mrs. MILLER. Yes, so I have another question for you Dr. Holtz-Eakin. Can you describe how negotiations with the Federal Government would work under H.R. 3? Would you describe their approach as a fair voluntary and market-based price negotiation?

Mr. HOLTZ-EAKIN. No I wouldn't. As I mentioned in my oral remarks, we looked at this issue when I was the Director of CBO, and it's been revisited many times in the years since. And if we position the secretary as we do a plan under Part D, the secretary couldn't do any better than that plan.

The secretary doesn't have a tool, doesn't have a formulary, doesn't have a way to move market share toward the manufacturer and thus get rewarded by a lower price. And so for that reason the CBO is included on a regular basis, there would be little impact of providing the secretary with negotiating authority.

This isn't that situation. It's not a market-based negotiation. This is a situation where there's a by fiat set a maximum price called the average international market price. And then "negotiations" begin where the secretary says I get to decide whether you're negotiating in good faith, or you're subject to a tax on 95 percent of your gross revenues, which is not deductible for income tax purposes, it is over 100 percent effective tax rate, and it would effectively close the domestic market to that manufacturer for that drug.

That's an enormously powerful government tax weapon being brought to the table, very different than a private negotiation.

Mrs. MILLER. Thank you and I yield back.

Chairman DESAULNIER. Thank you Representative. The Chair will recognize the gentlelady from Pennsylvania for five minutes, Representative Wild.

Ms. WILD. Thank you, Mr. Chair. Susan Wild here from Pennsylvania 7. I hope you can hear me OK. I am in a public place and therefore masked. My question is for Mr. Isasi, and it has to do with an issue that is incredibly important to the people in my district, and that is the cost of insulin.

We know that nearly a century ago the scientist who discovered insulin, rather than seek the profit from it, famously sold the patent for just one dollar as a gift to society. And for decades this lifesaving drug was available to consumers at a reasonable price.

Yet when I'm home in my district as I am now, and I'm talking to my constituents, I hear over and over again from patients who are finding that they are struggling to afford insulin. Mr. Isasi,

first of all what is going on here? Why are insulin prices increasing, and what is the cause?

And if you can comment also on how H.R. 3 would address these insulin prices, and ensure the consumers have access to this critical medication.

Mr. ISASI. Thank you so much for the question. Such an important one, we hear about it at Families USA all the time as you mentioned. About 30 million Americans across the country with diabetes, this is their life and death we're talking about. What we know is as you've said perfectly, insulin was developed over 100 years ago, over 100 years ago.

And the price is continuing to go up and up and up and up. Between 2002 and 2013 it tripled in price. A drug that's over 100 years old. What we see here is the classic manipulation on the part of drug manufacturers to make small changes to the drug to extend patents, extend patents, extend patents. That's the first thing that happens.

The other thing we know for sure is that drug makers like Sanofi and you know literally are working very hard to create patents that get around these drugs. They sue competitors, they stop the biologics from entering in. So this is a good example of the ways in which our system doesn't have any real competition period.

This is about abusing the legal system, smart lawyers, not innovative drugs. And insulin is a perfect example. And we see examples where literally people have died because they can't get their insulin right.

And so to your question about H.R. 3, this is a classic example where, as I've mentioned previously, our current health care system Medicare pays for hospital. Medicare pays for doctors. Medicare pays for devices. And Medicare negotiates a price. It sets a price every time.

But for some reason we have decided that drug companies shouldn't be subject to the same thing we do for the rest of the industry right. H.R. 3 would let us get in there and let the government sit down and negotiate drug prices on behalf of families to make sure that these families are not being saddled with tripling of prices of a 100-year-old drug.

Ms. WILD. Thank you so much. Mr. Chairman may I inquire how much time I have left?

Chairman DESAULNIER. Let me check. How much time does the Representative have left?

Ms. WILD. Thank you Mr. Chairman I yield back.

Chairman DESAULNIER. Thank you very much. I'm sorry. The Chair would now recognize the Ranking Member Mr. Allen for five minutes. Oh, I'm sorry. Mr. Fitzgerald are you back? Ms. Spartz you are after all the Subcommittee Members, so we would go to Mr. Allen first, then back to Ms. Stevens and then to Ms. Spartz. Mr. Allen you're recognized.

Mr. ALLEN. Great thank you Mr. Chairman. I agree this has been a great hearing and obviously, we really need to examine this from really all directions because you know my experience and Dr. Holtz-Eakin's I'm told, and I'd like you to give me some feedback on this.

But for example, you know back in the early 2000's people were complaining about healthcare costs period. And drugs, everything else. And of course we know today that we actually, this Nation pays about 10 times what any other nation pays for healthcare.

Now we also know that we have the best remedies and most innovative healthcare in the world, obviously by evidence of the development of this vaccine which nobody thought could possibly be done, and you can't imagine the millions of people who would not be with us today if that had not been done.

But you take going back to say the Affordable Care Act, you know the President said we will lower your premiums and we're going to lower your deductibles OK. And I understand that, and this is just the healthcare community talking to me out in my district.

I asked the question like so like for every premium dollar and every taxpayer dollar that goes into the Affordable Care Act, how much actually gets back to take care of a patient? And I was told maybe less than 20 percent. So this is my concern is when the government tries to control these things, the cost actually escalates, and the dollar value of healthcare goes to Washington, DC and not to the patient.

I mean for crying out loud, the Health and Human Services is what now, 1.4 trillion dollar organization. I mean help me with this. I mean is the government involvement in the Affordable Care Act driving some of these cost increases?

Mr. HOLTZ-EAKIN. So a couple things on that. First the real reason for premiums being higher is the underlying cost of care has gone up. We're spending a lot more on healthcare itself. Insurance is a means to spread that national healthcare bill around, but it's the bill itself that's the problem.

Of that bill, outpatient prescription drugs are 10 percent, and they've been 10 percent for a long time. It hasn't gone up at all. In-patient, particularly Part B has gone up somewhat in recent years, and these are these extremely innovative small market specialty drugs that I've mentioned before, and they are what stands out as something that Congress should think hard about looking at.

I think they are phenomenal medicine, but they are the ones where paying coinsurance on the list price for example is probably causing some financial pain. The real bulk of the bill is elsewhere, it's in doctor, and in hospitals and other providers.

That's where American spends its healthcare dollars, and one of the concerns that I would voice as an economist is that since the passage of the ACA we have seen considerable consolidation of the provider side of the market, and hospitals buying doctor groups, other consolidation.

That inhibits competition and leads to higher healthcare costs. I am concerned not just Federal, but State level certificate of need, scope of practice and a variety of other regulations are in fact getting in the way of lowering our healthcare bill and that's a problem.

Mr. ALLEN. And do you see the same thing with H.R. 3?

Mr. HOLTZ-EAKIN. As I think you probably gathered, I'm not a big fan of H.R. 3. It does nothing to improve market competition

in the United States. It does a lot to damage the futures of those who are faced with catastrophic illness, and I cannot support it.

Mr. ALLEN. Tell me about the messenger RNA and this innovative technology that developed the Pfizer and Moderna vaccines.

Mr. HOLTZ-EAKIN. So I think the past year is a fantastic, as I mentioned, a fantastic episode in public policy. But it was preceded by three decades of private risk capital attempting to develop this technology unsuccessfully, so this is where lots of money goes.

Moderna raised about 5 billion dollars of private capital. They weren't entirely publicly financed.

Mr. ALLEN. And this would not have happened had this been law.

Mr. HOLTZ-EAKIN. Right.

Mr. ALLEN. 10 years ago. OK.

Mr. HOLTZ-EAKIN. I wouldn't want to give them the money.

Mr. ALLEN. All right. Well thank you so much, and thank you for all of our witnesses we really appreciate this information and I yield back Mr. Chairman.

Chairman DESAULNIER. Thank you, Mr. Allen. It's always a pleasure. The Chair will now recognize Ms. Stevens, the gentlelady from Michigan.

Ms. STEVENS. Well thanks to our Chair for his phenomenal leadership, and dedication to this issue about lowering the cost of prescription drugs and also staying firm on what it means to ensure that every American has access to good affordable health care, something that we achieved when we passed the ACA into law, and saw it prevented it from being overturned in 2017.

Dr. Socal, first of all, thank you so much for your expertise and your wonderful testimony. As you know this past year has been devastating for our Nation's older adults who have been just clearly disproportionately impacted by COVID–19.

We're thrilled to see great rates of vaccinations among older Americans here in Oakland County we are at 80 percent of older Americans being vaccinated, but it's also an example why it is more important than ever to support the well-being of this population who is you know, just borne the brunt of the drug pricing crisis in our country for far, far, too long.

So much so that 1 in 5 older Americans report not taking their medication because of the cost. I have had people in my district, you know out at the farmer's market tell me such, that the costs of their prescription drugs are just too high, they're retired, they saved, they're living a fine retirement, but they can't pay $5,000 for arthritis medication.

So it's part of why I'm pleased to support H.R. 3, which is going to obviously improve innovation in our Medicare program to lower the cost of prescription drugs, and you know a provision that I led to improve the auto enrollment in Part D plans, so Dr. Socal, I'm just wondering, just based on your incredible research into older Americans drug pricing preferences, could you speak to how provisions in H.R. 3 will benefit older Americans in particular?

Dr. SOCAL. Absolutely, and it's such a big concern for this population right. So, first of all, older Americans under Medicare they don't have an out of pocket cap right now, and so when taking a drug, and I'll give an example from our research. Our No. 2 drug

in terms of top spending in Medicare Part D was a drug to treat cancer.

This drug was already in the U.S. market for 14 years when we did our research, and we in the U.S. we were paying 10 times more as Japan and three times more than the U.K. for this drug. This is just an example. Other drugs were similar. And the interesting part is that in our calculations if Medicare Part D were to use the average price around the country that we studied, just the savings from this price negotiation would amount to about $73 billion.

And I mention this number because you were talking about vaccination. $73 billion would be enough to vaccinate the U.S. population you know more than two or three times with COVID vaccines today, so it's an incredible amount of money that we are spending and disproportionately more than other countries for this population alone.

Ms. STEVENS. And you mentioned, Dr. Socal, in your testimony that you work with business organizations who are looking to lower the cost of health care in the United States, and I'd love to hear what you've learned through this work, you know, what are some of the challenges that businesses face in affording drugs and how does this impact the workers?

How would having a price negotiated by the Federal Government help these businesses, even though I know we kind of got into this a little bit already in the hearing.

Dr. SOCAL. Right. But there is a lot here and a lot of benefits, different benefits to different stakeholders. So, let's just take the same example that I used Revlimid. This is a drug that each pill of this drug costs about $600 in terms of list price right? Of course it's much less in terms of final negotiated price, but $600 per pill.

If we charge about 20 percent coinsurance to a patient who needs this drug, and this drug is used to treat cancer and it's a very important drug for those who need it. The coinsurance on each pill will amount to $120 that the person is going to be paying for each pill that they take.

So bringing up a benchmark and increasing transparency would—increasing transparency would lower the price in the out-of-pocket side for patients as well.

The other thing is in terms of 'me too' drugs, often times employees don't know that they're spending so much money. The price differentials between these 'me toos', and their corresponding generics, sometimes generics are 99.9 percent cheaper than the 'me too'. And that transparency would increase the knowledge about that in making decisions more informed, both from patients and their prescribers.

Ms. STEVENS. Thank you. I'm overtime and I know Dr. Mitchell too touches on this, so we'll probably just get you for written, but thank you to our Chair for this, I yield back.

Chairman DESAULNIER. Thank you, Ms. Stevens and no gold star, for you, but you could aspire in the future. Next speaker is the gentleman from Indiana Mr. Mrvan and then we'll go to our Chairman as of the moment and Mrs. Spartz. Representative Mrvan.

Mr. MRVAN. Thank you, Chairman. I appreciate this opportunity and I appreciate the panel being here and Ranking Member also.

In my previous elected position, I was responsible for assisting individuals who were having a hard time, or having an economic crisis paying for medications that were absolutely necessary for their health and the quality of their life.

Far too often they were being forced to make decisions on whether or not they could pay their rent or food, like Maureen, and pay for their medications. It was often a new and innovative drug that they needed in order to perform their work to support their family.

And they often needed that employment in order to have their health insurance. Mr. Mitchell and Dr. Socal, a recent study by the purchaser business group on health and the Kaiser Family Foundation found that more than 9 in 10 employers believe that the cost of providing health benefits is excessive. What impact does high health care costs have on the American businesses, and how can we move forward in a way that improves the ability for those most in need to have the life-saving, and sometimes career-saving medications?

Mr. MITCHELL. So Mariana do you want to go first? OK. About 56 percent of Americans get their health coverage through their employers. It's a huge amount of money that employers devote to health care in this country.

It turns out there was a recent survey, 77 percent of small business owners support direct Medicare price negotiation, and just yesterday the ERISA Industry Committee, which is large employers, said that they don't feel that H.R. 19 goes nearly far enough because the way it's structured is going to just shift costs onto employers because it doesn't lower drug prices.

So, employers around the country are hurting from the burden of high drug prices and trying to manage those, and H.R. 3 would extend the benefit of low prices into the private sector and help all Americans. Mariana, I hope I left time for you.

Dr. SOCAL. Let me just add one important thing. The reason why employers are so crucial to this conversation is not only because of the price of the drug. When the patient cannot afford the drug that they need, we already discussed this, one of the consequences is that they're going to increase their medical costs. People are going to start, you know, missing days at work.

And employers are the ones who are going to foot the bill for these increased medical costs. So imagine a person taking insulin, if they cannot afford to take their insulin that particular month, they are going to need, frequently you know, a visit to the emergency department, a visit to the hospital.

They're going to need perhaps a hospitalization. Not to mention the long-term consequences from the lack of access to their insulin. And spending on those services is going to be paid by the employers. So it's a very important goal to make sure that affordability of drugs such as insulin is met in these cases.

Mr. MRVAN. And I just want to reiterate what I'm taking away is the non-compliance based on economical reason have a cost on health care. And ultimately, the next question I have for Dr. Socal, and I want to get my gold star, is what studies, or what information do you have about the choices that older Americans may make when it comes to pricing and medications?

Dr. SOCAL. Right. Well we know that 3 out of 10 Americans have struggled to pay for their prescription drugs, right? And one in these 3 they have experienced a worsening of their condition because of that.

And earlier we talked about the fact that you know so far we have assumed that the government not negotiating in Medicare was not supported, but there is very strong public support for the government to negotiate. And 60 percent of older Americans would tradeoff the option to you know, choose or change their drug, prescription drug plan in Medicare Part D, if they were to get lower drug costs.

So there is very strong support for that.

Mr. MRVAN. I thank you very much and I appreciate your testimony today and I yield back to the Chairman.

Chairman DESAULNIER. Thank you. I'm afraid I might be inhibiting good questions, using gold stars as a motivator, but thank you for being timely. I have as the next speaker, the Chairman, Mr. Scott, you are recognized for five minutes, and then we'll go to Congresswoman Sparks.

Mr. SCOTT. Thank you. Mr. Holtz-Eakin you mentioned increasing supply as a strategy to reduce prices. How do you pull that off with drugs that are protected by patents? How do you increase supply?

Mr. HOLTZ-EAKIN. Well certainly there is competition between on patent branded drugs. I mean the poster child for that would be Sovaldi and then the competitors that came on and forced dramatic price reductions in that drug. I think that's the most recent example to point to.

In terms of ways that you do that, you certainly want to streamline the approval process to the extent possible. You want to make sure that you have an effective and regular process for the authorization of generics and biologics. Those are things that I think of that have been an extraordinarily valuable contribution to American medicine, and are cheaper in the U.S. than around the globe.

And I think making sure that those processes are understood, certain, and executed the same for every drug is very important.

Mr. SCOTT. Thank you. Mr. Mitchell we're talking about negotiating at a price at 120 percent the average international market price. At our last hearing we were told that if it's 120 percent that would mean that the biggest market, the United States, would be paying the highest price, so we wouldn't have to worry about people pulling out of the market.

But Mr. Holtz-Eakin did accurately cite the CBO report that said that there would only be a negligible affect if all we did was granted negotiation. We would have to do more than that. What more would we have to do to get meaningful reduction in pricing along with negotiation?

Mr. MITCHELL. Well we'd have to have an incentive for the drug companies to engage in negotiations because if there is none, then there will be no savings. You know folks today have talked about using international referenced pricing as fiat. Well this fiat is supported and was proposed by the former President of the United States who is still as near as I can tell the leader of the republican party.

And he believes that we should use international referenced pricing not to lower prices, but to get the lowest price of any nation in the world. So I would say that international reference pricing is one of the things that we can use consistent with former President Trump in order to help set a boundary for negotiations.

And then we have to have a stick to go with the carrot when we negotiate, both. And by the way carrots and sticks are frequently used in negotiations. Both of those things.

Mr. SCOTT. Thank you. And Dr. Socal a lot of concern has been raised about the fact that if this bill passes there will be great savings, in the hundreds of billions of dollars, money that could have gone to research and therefore there will be fewer drugs created.

Isn't it true that if we set aside a portion of those savings to research and the NIH we could offset that loss?

Dr. SOCAL. Absolutely. The FDA is contributing to every single drug that is approved currently, to the drug development, and you know the most frequently prescribed drugs in America, 93 percent of them have had NIH funding.

What the H.R. 3 mechanism of negotiation would possibly do is reduce innovation, so-called innovation for these 'me too' drugs. But for the really effective drugs we would still have the NIH research pushing for these innovative routes, the innovating mechanisms of actions for really innovative drugs.

Mr. SCOTT. Well thank you. And Mr. Isasi if this bill passes there's a suggestion earlier that there would be a lot of consolidation in the industry. Did you want to comment on that?

Mr. ISASI. All right, sure. So, I think fundamentally the question is Dr. Holtz-Eakin mentioned that the industry's business model would change. It would stop abusing pricing and their market shares would go down.

But somehow there would be a lot of people interested in merging together, but the two things don't make any sense. This bill does not increase consolidation in a pharmaceutical industry. That's already happened. You see massive consolidation in the pharmaceutical industry already.

What this bill does is say in a very distorted market where there are no real negotiations going on, and a lot of abuse, the government is going to get in there and make sure that the American people are getting a fair price.

Mr. SCOTT. To my great surprise, Mr. Chair. I yield back.

Chairman DESAULNIER. Mr. Chairman you always get a gold star. All right. The Chair will now recognize the representative from Indiana, welcome to the Subcommittee, Mrs. Spartz you have the floor for five minutes.

Ms. SPARTZ. Well thank you Mr. Chairman. I appreciate the time and appreciate the discussion. I share the frustration of a lot of Members of this Committee. I would probably argue that price and wage controls after World War II, that's what got us in trouble with healthcare.

And there are some issues that in this bill probably would be great in informing the FDA, dealing better with substance abuse, better negotiation for Medicare for seniors, PBM transparency, you know we need to deal with hospital care, which was over 50 per-

cent of spending, and have really inflation, hyper-inflation of price I would argue.

We also dealt with some issue in the Subcommittee that we have to deal, but I have a major concern with House Bill 3. And I'll tell you the major concern I have and in particular dealing with we're given enormous power to HHS Secretary to have a very, you know, very powerful tax weapons to have the requirements to do government reporting and collecting of data, and also you know creating models to enforce mechanism of government price control for the group market.

And if you look at the group market you know it's about like in the State of Indiana, 90 percent of the private payers market. So my question is to Dr. Holtz-Eakin. What do you think if we're going to have you know dominant control, and dominant takeover, over 90 percent of you know private payer market, that would be you know something like almost maybe not in its form, but in substance, almost like nationalization of the pharmaceutical market, and a little bit closer to Communist China in our control than being a free enterprise country.

I mean isn't it like if you look at that, that is a powerful control and centralization of power of the Federal Government which I understand government can control price, and we can do very good, but it's going to be you know fatal for innovation.

Mr. HOLTZ-EAKIN. You've said my concern very succinctly, and this is a sweeping, sweeping proposed legislation. I mean it would take over an enormous swath of the pricing of pharmaceuticals in the United States. It would do it in a way that I think is at odds with providing appropriate incentives for innovation.

There's always a tradeoff in a situation with patents, so between incentives for innovation and a rival market competition, I think this goes way too far in the wrong direction and would damage the ability of future citizens to have access to therapies and innovations they need.

Mrs. SPARTZ. If you would just kind of put out what would be three key things that you think would be actually beneficial that would have more competition on the market, versus alternative big government monopoly? Because ultimately, we're providing alternatives to oligopoly would be created for a lot of reasons, and I'm not going to debate here.

We're giving the alternative of ultimately government monopoly on the market. What would be a better solution if you could highlight maybe three issues that we could do better.

Mr. HOLTZ-EAKIN. Sure. I think three things that I would highlight are No. 1, one of the real poster child of the price increase of pharmaceuticals has really been sole sourced generics, not branded drugs.

And to make sure that we do not get abusive market power with sole sourced generics I think would be something that we've got to make sure that the FTC and the DOJ are effectively dealing with. So that could be No. 1.

No. 2: Use the levers that you can pull, and you can redesign the Part B benefit to protect beneficiaries from catastrophic costs to improve the incentives to negotiate effectively for lower prices be-

tween plans and manufacturers, and to save the taxpayers some money.

I'd like to see that. That could spill over as a result to the broader pharmaceutical market because it's a quarter pharmaceutical spending, so that's important.

And No. 3 one of the painful situations that arises again and again is this disparity between coinsurance calculated on list prices, and then net price that has been negotiated by a PBM or by a plan. And Congress could eliminate that disparity by passing the rebates through to the retail lever.

It hasn't done that. That's a rifle shot compared to the sweeping thing you have under consideration, but would solve a lot of the problems that you hear about.

Mrs. SPARTZ. Thank you. And I hope we can agree on some issues and work on some other issues that in a bipartisan way, but I appreciate this discussion, and I appreciate being part of the Subcommittee and I yield back.

Chairman DESAULNIER. Thank you so much for joining us. That is the last person that we have questions for. So, I want to thank the panel again, really wonderful discussion. I appreciate the content and the tone to a very vexing problem.

I want to remind my colleagues that pursuant to Committee practice materials for submission for the hearing record must be submitted to the Committee Clerk within 14 days following the last day of the hearing, so by close of business on May 19, 2021, preferably in Microsoft Word format.

The materials submitted must address the subject matter of the hearing. Only a Member of the Subcommittee or an invited witness may submit materials for inclusion into the hearing record. Documents are limited to 50 pages each. Documents longer than 50 pages will be incorporated into the record via an internet link that you must provide to the Committee Clerk within the required timeframe please.

But please recognize also, that in the future that link may no longer work. Pursuant to House rules and regulations items for the record should be submitted to the Clerk electronically by emailing submission to *edandlabor.hearings@mail.house.gov*.

Again, I want to thank the witnesses, really terrific. Good spirited debate and information that helps inform our policymaking. Members of the Subcommittee may have some additional questions for you, and we ask the witnesses to please respond to those questions in writing. The hearing record will be held open for 14 days in order to receive those responses.

I want to remind my colleagues that pursuant to Committee practices witness questions for the hearing record must be submitted to the Majority Committee Staff, or the Committee Clerk within 7 days. The questions submitted must address again the subject matter of the hearing.

I now want to recognize the distinguished Ranking Member for closing statement, Mr. Allen.

Mr. SCOTT. I think you're on mute.

Chairman DESAULNIER. Mr. Allen are you there?

Mr. SCOTT. He's there but he's having a technical difficulty. There he is.

Mr. ALLEN. Thank you Mr. Chairman. I appreciate that.

Chairman DESAULNIER. There we go. The floor is yours.

Mr. ALLEN. Sorry about that.

Chairman DESAULNIER. That's OK.

Mr. ALLEN. Mr. Chairman thank you for this hearing today. It's shed a lot of light on this subject. I do ask the unanimous consent to enter into the record a statement from the National Association of Healthcare Underwriters raising concerns with H.R. 3.

Chairman DESAULNIER. Without objection.

Mr. ALLEN. Thank you Mr. Chairman. And again I want to thank all the witnesses for their testimony today. Me and my colleagues today have implied that NIH or the Federal Government invented the Pfizer and Moderna vaccines, or worse that the U.S. Government has ownership or co-ownership of vaccines through patents.

These claims are blatantly false. If H.R. 3 were law before the pandemic, the speed and development of Moderna and Pfizer vaccines would likely not have been possible. Today over 100 million adults are vaccinated against COVID–19. This is a life saving scientific technological and logistical feat unheard of in the history of medicine.

Again I will remind my colleagues that many Americans are facing skyrocketing healthcare costs because of dramatic increases and out of pocket costs of prescription drugs. And we need to do something about it.

In 2018 patients paid a collective 61 billion in out of pocket drug costs, and we need to come together on a bipartisan basis and promote legislation that will solve this problem, but not a war on you know the healthcare industry, like we had a war on fossil fuels that now the price of gasoline is almost doubled.

So fortunately, republicans are stepping up with solutions that work best for the people without the heavy hand of government interference. H.R. 19, the Lower Cost More Cares Act introduced last month by the republican Committee leaders is exact opposite of H.R. 3.

This is Speaker Pelosi's big government power grab. The Lower Cost More Cares Act will utilize the power of the free market to modernize our healthcare system, increase choice and transparency and lower costs. These are goals that both sides of the aisle should be able to rally behind, and the American people demand it.

At the end of the day H.R. 3 would only lead to fewer treatments and cures, decreased competition in the marketplace and increased reliance on Communist China. Rather than promote partisan socialist policies such as H.R. 3, I again urge my colleagues to work together on finding a bipartisan solution for lowering drug costs like the common sense provisions included in H.R. 19.

Again, what we're talking about here is a government takeover of the pharmaceutical industry through H.R. 3. Again I thank you and the witnesses for participating today, and with that Mr. Chairman thank you again, and I yield back.

Chairman DESAULNIER. Thank you, Mr. Allen. I appreciate your comments although we do have a respectful disagreement. I don't see this as a government takeover. As a former small business person, as somebody whose met a payroll many times like yourself,

this is the challenge of a mixed market where we do have a good partnership with the private sector, but transparency is important.

And a reasonable rate of return that incentives investment from the private sector, but still acknowledges the value of taxpayer's investment I think is important to the indiscriminate bystander. So, to that, any of the witnesses, and thanks again to a terrific panel, to all of you. If you have information that helps to quantify this, where the value is and what the incentives are for appropriate Dr. Holtz-Eakin, what I was trying to get as a reasonable rate for return, is also an acknowledgement of what's high risk and what's high return.

At a hearing we had on oversight when Chairman Cummings was leading this, and his memory, I constantly think of him when we go through this, and the personal discussions we had about our health conditions.

Is it should be high risk, high reward, not low risk, high reward. So somewhere in there and Dr. Holtz-Eakin and other panelists, if you can inform me personally as one Member of Congress, as to knowing this isn't a perfect science, but at least to further quantify how we incentivize that partnership and get it right.

You know and I appreciate the comment about price control post-World War II, but my reading of history is price control helped contribute to us winning World War II and liberating Europe. So there's a balance here that we're looking for and I appreciate the respectful disagreement we have, and somewhere in there having a meeting of the minds that helps to remedy the situations that people like myself and Mr. Mitchell and other people who have testified to this and other Members, is getting that right incentive. I know I had a meeting with my oncologist yesterday, and my cancer is fighting to find a way back to health, so we're looking at other treatments.

So, this is the right, sort of the right temperature of the porridge if I can use that metaphor. So, I appreciate all of this and look forward to ongoing discussion. Again, I want to thank the witnesses. I want to thank my colleagues for really, I thought, a very helpful hearing.

And I will go purchase some gold stars so I can get a real reward. Pavlov's dog applied to the Subcommittee. Today's hearing to me reaffirmed the urgent need for the Federal Government to reduce and contribute to reducing the cost of prescription drug prices. All this innovation is only helpful if people can access that innovation, and there's a reasonable rate of return for the investor.

As we heard from compelling witnesses, Americans across the country are struggling to access prescription drugs they need to be healthy, while also weathering this pandemic. No better time to have some solutions to this problem in my view.

Yet we know that prescription drug companies are not charging exorbitantly high prices because of purely natural market forces, or because they are investing in research and development, although that is true to a degree.

Instead, drug companies are inflating prices simply because they can. Not all drug companies, but too many in my view. It's time for price gouging of America's consumers at the pharmacy counter to end. And a transparency for the consumer to flourish. As we con-

tinue to recover from the worst public health emergency in our history, our recent history for certain, we should be doing everything we can provide consumers with relief from the weight of unaffordable drug prices.

Simply put, medicines are of no use if those that need them can't afford them. In the months ahead I look forward to working with my colleagues to finally pass the Elijah E. Cummings Lower Drug Costs Now Act, and help ensure that people can get the medication they need to stay healthy and thrive, and continue America's leadership on this issue.

If there's no further business without objection I want to thank you all again, the Subcommittee stands adjourned.

[Additional submissions by Mr. Allen follow:]

Statement for the House of Representatives Subcommittee on Health, Employment, Labor, and Pensions

May 5, 2021

Lower Drug Costs Now: Expanding Access to Affordable Health Care

Submitted by
National Association of Health Underwriters

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I am writing on behalf of the National Association of Health Underwriters (NAHU), a professional association representing over 100,000 licensed health insurance agents, brokers, general agents, consultants and employee benefits specialists. The members of NAHU work daily to help millions of individuals and employers of all sizes purchase, administer and utilize health plans of all types. Our members have daily first-hand experience with how Americans are struggling with pharmaceutical prices. As such, we have a great interest in legislation that could lower prescription drug prices and reduce out-of-pocket costs for patients.

Prescription drug prices in the United States are significantly higher than 32 other nations, averaging 2.56 times greater for generic drugs and 3.44 times greater for brand-name medications. [1] These high drug costs have forced consumers to make difficult choices, like spending less on groceries, putting off a doctor's visit, or even declining to fill a necessary medication prescribed by their physician.[2] Accordingly, NAHU believes that extensive public policy action needs to occur to reduce the cost of prescription pharmaceuticals in the United States. Our comments today are primarily focused on H.R. 3, also known as the Elijah E. Cummings Lower Drug Costs Now Act.

One of the main provisions of H.R. 3 would allow the the secretary of Health and Human Services to negotiate prescription drug prices for the Medicare program and its beneficiaries. The secretary would be required to negotiate a minimum of 25 drugs in 2023 and at least 50 drugs in 2024, with international prices from six comparable high-income nations (Australia, Canada, U.K., France, Germany, and Japan) determining the minimum and maximum prices for the negotiation process. The minimum price would be the lowest price of the drug in any of those six nations, while the maximum price would be 120 percent of the average international market price across the comparable countries. Following negotiation with Medicare, H.R. 3 would extend this negotiated price to commercial plans as well.

Regarding an international pricing index, the Trump Administration proposed a similar system of international reference pricing to cap rates paid for physician-administered drugs under Medicare Part B. This regulation was structured as a seven-year Medicare demonstration project and as a mandatory model tying U.S. prices for affected drugs to those paid in a group of developed countries. This regulation, unlike H.R. 3, would only apply to Part B prices.[3]

NAHU, generally speaking, supports granting the Medicare program the ability to negotiate with pharmaceutical companies. Many comparable countries already enter such a negotiation process. According to a 2019 CBO analysis, these negotiation provisions, if enacted, would lower spending by about $456 billion.[4] However, NAHU has several concerns in this area, including possible negative impacts of price-setting on innovation and the potential for cost-shifting from Medicare to commercial plans.

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[1] Mulcahy, Andrew W., et al, International Prescription Drug Price Comparisons: Current Empirical Estimates and Comparisons with Previous Studies. Santa Monica, CA: RAND Corporation, 2021.
[2] Gill, Lisa. The Shocking Rise of Prescription Drug Prices. Consumer Reports. 26 November 2019.
[3] Centers for Medicare and Medicaid Services. Most Favored Nation (MFN) Model. 27 November 2020. Accessed 3 May 2021.
[4] Congressional Budget Office. H.R. 3, Elijah E. Cummings Lower Drug Costs Now Act. 10 December 2019.

The CBO recently released a report analyzing the research and development (R&D) process in the pharmaceutical market, where they noted that the amount of money that is devoted to R&D is directly determined by the projected revenue from a new drug and policies that influence the supply and demand for said drugs.[5] When the anticipation of future profits is higher, companies tend to invest more in R&D and produce more new drugs, but if expectations about prices and profits are lower, companies tend to invest less in R&D, ultimately leading to fewer drugs developed. By capping the price of certain high-cost drugs at 120 percent of the average international market price, regardless of any changes in demand, NAHU is concerned that H.R. 3 will lead to a decrease in R&D spending, a decrease in innovation and therefore a decrease in the number of drugs that American consumers have access to.

H.R. 3 would also penalize manufacturers that do not comply with these provisions, subjecting them to an excise tax ranging from 65 percent to 95 percent of gross sales of the drug. Because of this penalty, in combination with establishing a maximum potential price and then extending these prices to commercial plans, NAHU is uncertain whether the negotiation process outlined in this bill may effectively lead to the federal government setting prices in the private market. Price-setting can be a dangerous precedent to set in this area, and we are apprehensive about potential ramifications of these price controls and the implications it has for regulation of the health insurance market moving forward.

We would like to direct the committee's attention to the German negotiation model. For context, most German citizens obtain health insurance from one of 110 competing, non-governmental "sickness funds," with the premiums paid by employers and employees and with governmental subsidies for the unemployed and retired. These nonprofit health plans participate in the national system of employer-sponsored payment, risk adjustment, and centralized price negotiations with physicians, hospitals, and pharmaceutical manufacturers.[6] In Germany, health plans reward innovative drugs that provide genuine clinical breakthroughs through a clinical comparative effectiveness review by the non-governmental nonprofit Institute of Quality and Efficiency in Healthcare (IQWiG). If the IQWiG determines that a new drug provides a major or considerable added benefit for patients, then that sets the basis for negotiations with the Federal Joint Committee, a private organization governed by the associations of sickness funds, physicians, hospitals, and patient advocates. This classification allows manufacturers to get higher prices for more innovative drugs.[7]

If parties cannot reach agreement in the negotiation, the drug's price is reviewed by an arbitration panel with representatives from both entities, who reach a decision based on comparable international prices. And in the interest of price transparency, every negotiated or arbitrated price is public knowledge, so consumers understand the process used to determine the price.[8] While we are not suggesting that the U.S. adopt every aspect of the German model, it is important to understand how their system manages to keep prices lower than American prices without stifling innovation in the process.

[5] Congressional Budget Office. Research and Development in the Pharmaceutical Industry. April 2021.
[6] The Commonwealth Fund. Drug Price Moderation in Germany: Lessons for U.S. Reform Efforts. 23 January 2020.
[7] Lauterbach, Karl, et al. Germany's Model For Drug Price Regulation Could Work In The US. *Health Affairs*. 29 December 2016.
[8] National Coalition on Health Care. National Coalition on Health Care Policy Priorities U.S. Drug Pricing and the German Model. 7 August 2020.

Another major provision of H.R. 3 is the creation of "reverse price hikes," new inflation rebates that would apply to over 8,000 prescription drugs available in Medicare Part B and D. This would limit annual price increases for drugs covered under Part B and under Part D to the rate of inflation as measured by the consumer price index. We appreciate the logic behind this proposal, since drug companies have hiked the costs of drugs well beyond the rate of inflation, even for drugs that have been on the market for some time. One prominent example of this is the life-saving EpiPen, an allergy shot used during serious allergic reactions; the drug company Mylan increased the EpiPen's cost from roughly $60 in 2007 to over $700 in 2016 for a pack of two units. Additionally, a precedent has already been set regarding capping certain drug prices in Medicare. Several NAHU members who work with Medicare beneficiaries have reported positive feedback regarding the new $35 insulin price cap in certain Medicare plans. Per CMS estimates, Medicare beneficiaries who use insulin and join one of the participating plans could see an average out-of-pocket savings of $446 per year, or 66 percent.[9]

However, we still harbor concerns with the inflation rebates outlined in H.R. 3. By tethering this provision to the consumer price index alone, this stipulation essentially caps drug prices without consideration for R&D and manufacturing costs. As was mentioned earlier, the amount of money that a manufacturer devotes to R&D is directly determined by the projected revenue from a new drug and policies that influence the supply and demand for the drug. If an economic event occurs that has a drastic impact on drug manufacturing costs, then there may be a legitimate market-based rationale to why a drug price may increase beyond the rate of inflation in a short period of time.

Furthermore, NAHU is concerned that this would result in cost-shifting, ultimately increasing prices for those on commercial plans. On the medical side, Medicare sets reimbursement rates lower than private payers and the costs are shifted to the private market; since Medicare pays providers an average of 80 percent of the cost of care delivered,[10] providers routinely make up for this short-fall by charging private plans more.[11] Any legislation aimed at lowering drug costs must consider the potential for this cost-shifting to occur in the pharmaceutical market as well.

In addition to these major provisions, H.R. 3 would also establish a hard cap on out-of-pocket spending for Medicare beneficiaries that would initially be set at $2,000 per year. For catastrophic costs, the proposal would reduce Medicare payments from 80 to 20 percent, increases plans' share from 15 to 50 percent, and requires drug manufacturers to pay that 30 percent difference. H.R. 3 would also phase out the coverage gap and require manufacturers to pay 10 percent of costs. Once again, a primary concern of ours is the potential for plans to make up the lost revenue by shifting the cost from the Medicare market to the individual and employer markets.

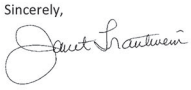
While we have reservations about many provisions in H.R. 3, there are already proposals in existence that NAHU supports that would eliminate anti-competitive practices and ensure a freer, fairer market. For example, eliminating "pay-for-delay" deals between pharmaceutical companies in which one company pays a generic competitor to delay research, production, or sale of a competitive drug. The FTC estimates that ending these pay-for-delay agreements

[9] Centers for Medicare and Medicaid Services. President Trump Announces Lower Out of Pocket Insulin Costs for Medicare's Seniors. 26 May 2020.
[10] Centers for Medicare and Medicaid Services. How to Use the Searchable Medicare Physician Fee Schedule (MPFS). March 2021.
[11] Milliman. Why hospital cost shifting is no longer a viable strategy. June 2010.

would save $3.5 billion each year for patients, insurers, and government programs.[12] This provision is included in the Protesting Consumer Access to Generic Drugs Act, which is also being considered during this hearing. This could level the playing field in the pharmaceutical market, and allowing for increased competition earlier in the lifespan of a drug may decrease costs to the point where many of the issues H.R. 3 seeks to address may be resolved.

We appreciate the opportunity to provide these comments and would be pleased to respond to any additional questions or concerns of the committee. If you have any questions about our comments or if NAHU can be of assistance as you move forward, please do not hesitate to contact me at either (202) 595-0639 or jtrautwein@nahu.org.

Sincerely,



Janet Stokes Trautwein
CEO, National Association of Health Underwriters

[12] Federal Trade Commission. Pay for Delay. Accessed 3 May 2021.

CHAMBER OF COMMERCE
OF THE
UNITED STATES OF AMERICA

NEIL L. BRADLEY
EXECUTIVE VICE PRESIDENT &
CHIEF POLICY OFFICER

1615 H STREET, NW
WASHINGTON, DC 20062
(202) 463-5310

May 4, 2021

TO THE MEMBERS OF THE U.S. HOUSE OF REPRESENTATIVES:

The U.S. Chamber of Commerce strongly opposes H.R. 3, the "Lower Drug Costs Now Act of 2019." This legislation would impose government price controls on prescription drugs, which threatens to cut critical medical research dollars essential for innovation and development of new cures while endangering the livelihood of nearly 1 million Americans. **Members who do not cosponsor this legislation will receive credit for the Leadership component of the Chamber's "How They Voted" legislative scorecard.**

The harmful proposals included in H.R. 3 would limit access, increase costs for employers and workers, and inhibit innovation. Economic analysis on the legislation support these assertions. The Congressional Budget Office has previously concluded that H.R. 3 would result in approximately eight fewer drugs being introduced to the U.S. market over the 2020-2029 period, and about 30 fewer drugs over the subsequent decade. Those 30 drugs represent approximately 10% of expected new drugs that would otherwise come to market. Research has also found that implementing international reference pricing in the United States would:

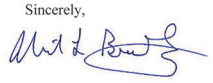
- Cut earnings by 62% on average for affected companies, with one third (32%) of affected companies having reductions larger than 95% of earnings.
- Diminish biopharmaceutical companies' investments in smaller company R&D through M&A, partnerships and other arrangements.
- Reduce by 90%+ the number of medicines developed by small and emerging biotechs, resulting in 61 fewer medicines over 10 years.
- Disproportionately impact new treatments in rare diseases, oncology, and neurology.
- Create large investment ecosystem losses to smaller companies in 19 states.
- Eliminate nearly 200,000 biopharmaceutical industry jobs, and nearly 1 million jobs across the economy.

Rather than advancing proposals that will curb access to lifesaving medicines and eviscerate an estimated 1 million American jobs, policymakers should work to address the problems in our current system by taking the following steps:

- Consider ways to rework and reform the Medicare Part D benefit design;
- Streamline the innovator to generic transition;
- Help reduce costs for all Americans by moving towards a more value-based system that rewards outcomes and limits costs;
- Help Americans with out-of-pocket costs through expansion of Health Savings Accounts and Health Reimbursement Arrangements;
- Solidify the ACA's exchanges through the use of risk corridors and funding cost-sharing reduction payments.

The Chamber looks forward to working with Congress on legislation that improves access to and reduces the cost of healthcare, and which preserves the free market and the employer-sponsored insurance system.

Sincerely,



Neil L. Bradley

NATIONAL ASSOCIATION OF Manufacturers

Robyn Boerstling

Vice President

Infrastructure, Innovation and Human Resources Policy

May 4, 2021

The Honorable Nancy Pelosi
Speaker of the House of Representatives
Washington, DC 20515

Dear Speaker Pelosi:

On behalf of the National Association of Manufacturers, the largest manufacturing association in the United States representing 14,000 manufacturers in every industrial sector and in all 50 states, I am writing to convey strong opposition to H.R. 3, legislation that would deter future medical innovation and result in a hidden tax on manufacturers.

The NAM opposed H.R. 3 in 2019 and continues to oppose the bill today as it runs counter to many of the core economic principles that drive the American economy. Global medical innovation is inherently reliant on the market-based health system we have established. Far more vaccines and treatments are available in America than in any other nation. And we benefit from a unique innovation ecosystem of hospitals, universities and drug manufacturers that seek to advance the latest research and therapies for patients of all ages affected by various diseases, including some of the hardest to cure and the most threatening to public health.

At the same time, health care costs have been rising too quickly for far too long for American families. While everyone agrees with the goal of reducing the costs of health care, manufacturers believe that the values that have made America exceptional—free enterprise, competitiveness, individual liberty and equal opportunity—must guide that process. Effective solutions will require a different kind of approach to thwart the various forces that strain the system. Innovation can and should be used as a tool to reduce costs and improve health outcomes. With that in mind, we oppose the price-setting called for in H.R. 3.

While well-intentioned, an orientation toward price controls would abandon the market-based core principle of the Medicare Part D program and act as a hidden tax on both manufacturers and innovation. Accordingly, a past Congressional Budget Office analysis found that H.R. 3 would result in reduced investments in research and development and fewer new, innovative drugs introduced to the U.S. market. Price controls of any kind are never a correct solution as they restrict supply, discourage competitiveness and undermine the free enterprise system that forms the bedrock of our economy and way of life. We should not employ the anti-competitive or restrictive practices of our overseas counterparts in the name of health care reform.

The NAM opposes H.R. 3 and encourages Congress to instead focus its efforts on steps to improve efficiency, affordability and transparency by focusing on measures that will drive down health care costs without abandoning market-based approaches.

Sincerely,

Robyn M. Boerstling

PILMA
Pharmaceutical Industry
Labor-Management Association

2021 Statement on H.R. 3

April 23, 2021

The Pharmaceutical Industry Labor-Management Association (PILMA), a coalition of biopharmaceutical companies and skilled construction labor unions, reiterate our grave concerns with H.R. 3, the recently announced drug pricing proposal.

This short-sighted legislation would decimate the U.S. biopharmaceutical industry at a time when vaccines and therapeutics developed by this industry have been instrumental to navigating our way out of the COVID-19 pandemic. The unintended consequences of this bill would be catastrophic for union construction jobs, halting billions of dollars in infrastructure investment and laying off union workers that depend on high-quality jobs provided by the industry for their livelihoods.

The U.S. biopharmaceutical industry supports more than 4.7 million American jobs and is responsible for the largest share of business research and development in the U.S. economy. Research shows this bill would eliminate nearly 1 million jobs in the U.S.

PILMA remains fundamentally opposed to price control measures. H.R. 3 gives the government unprecedented control over price setting of medicines in both public and private markets. This would upend the market-based system that has incubated the development of innovative medicines and vaccines, and drive jobs overseas.

During this worldwide pandemic, when the biopharmaceutical industry and its union partners have made extraordinary strides in developing vaccines and therapies to eradicate the COVID-19 virus and enable our economy to bounce back, severe and punitive price control measures are especially ill-advised. Under the original Congressional Budget Office analysis of H.R. 3, it was estimated that fewer new drugs would come to market under these provisions.

H.R. 3's outcomes would result in reduced medicine availability in U.S. markets, which is of critical concern amid the worldwide vaccination effort. Many countries are now prohibiting exports of vaccines and the components required to produce them. Without research and manufacturing operations located here in the U.S., we will no longer be able to protect and provide Americans with the quality healthcare delivery system we have come to rely on.

The U.S. develops more medicines than the rest of the world combined because of its strong protections of intellectual property rights and free market economy. Carving out these pillars of our democracy would be a grave mistake for the future of innovation and resulting new medicines, jobs, and economic health.

[Additional submission by Mr. Courtney follow:]

United States Patent **10,960,070**
Graham , et al. **March 30, 2021**

Prefusion coronavirus spike proteins and their use

Abstract
Coronavirus S ectodomain trimers stabilized in a prefusion conformation, nucleic acid molecules and vectors encoding these proteins, and methods of their use and production are disclosed. In several embodiments, the coronavirus S ectodomain trimers and/or nucleic acid molecules can be used to generate an immune response to coronavirus in a subject. In additional embodiments, the therapeutically effective amount of the coronavirus S ectodomain trimers and/or nucleic acid molecules can be administered to a subject in a method of treating or preventing coronavirus infection.

Inventors: **Graham; Barney** (Rockville, MD), **McLellan; Jason** (Austin, TX), **Ward; Andrew** (La Jolla, CA), **Kirchdoerfer; Robert** (La Jolla, CA), **Cottrell; Christopher** (La Jolla, CA), **Joyce; Michael Gordon** (Washington, DC), **Kanekiyo; Masaru** (Chevy Chase, MD), **Wang; Nianshuang** (Hanover, NH), **Pallesen; Jesper** (La Jolla, CA), **Yassine; Hadi** (Doha, **QA**), **Turner; Hannah** (La Jolla, CA), **Corbett; Kizzmekia** (Bethesda, MD)

Applicant:

Name	City	State	Country	Type
The United States of America, as represented by the Secretary, Department of Health and Human Services	Bethesda	MD	US	
The Scripps Research Institute	La Jolla	CA	US	
Trustees of Dartmouth College	Hanover	NH	US	

Assignee: **The United States of America, as represented by the Secretary, Department of Health and Human Services** (Bethesda, MD)
The Scripps Research Institute (La Jolla, CA)
Trustees of Dartmouth College (Hanover, NH)

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PCT Pub. Date: **May 03, 2018**

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Related U.S. Patent Documents

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Application Number	Filing Date	Patent Number	Issue Date
62412703	Oct 25, 2016		

Current U.S. Class:	**1/1**
Current CPC Class:	A61P 31/14 (20180101); A61K 39/12 (20130101); A61K 39/215 (20130101); C07K 14/005 (20130101); C12N 7/00 (20130101); C12N 2770/20034 (20130101); C12N 2770/20022 (20130101); C12N 2770/20071 (20130101)
Current International Class:	A61K 39/215 (20060101); C07K 14/005 (20060101); A61P 31/14 (20060101); C12N 7/00 (20060101)

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Primary Examiner: Salvoza; M Franco G
Attorney, Agent or Firm: Klarquist Sparkman, LLP

Parent Case Text

CROSS REFERENCE TO RELATED APPLICATIONS

This is the U.S. National Stage of International Application No. PCT/US2017/058370, filed Oct. 25, 2017, which was published in English under PCT Article 21(2), which in turn claims the benefit of U.S. Provisional Application No. 62/412,703, filed Oct. 25, 2016. The provisional application is herein incorporated by reference in its entirety.

Claims

It is claimed:

1. An immunogen, comprising: a recombinant coronavirus S ectodomain trimer comprising protomers comprising one or two proline substitutions at a junction between a heptad repeat 1 (HR1) and a central helix that stabilize the S ectodomain trimer in a prefusion conformation.

2. The immunogen of claim 1, wherein the recombinant coronavirus S ectodomain trimer comprises two consecutive proline substitutions at the junction between the HR1 and the central helix.

3. The immunogen of claim 1, wherein the coronavirus is one of MERS-CoV, SARS-CoV, NL63-CoV, 229E-CoV, OC43-CoV, HKU1-CoV, WIV1-CoV, MHV, HKU9-CoV, PEDV-CoV, or SDCV.

4. The immunogen of claim 1, wherein the coronavirus is a betacoronavirus.

5. The immunogen of claim 1, wherein the protomers of the recombinant coronavirus S ectodomain trimer further comprise one or more additional amino acid substitutions that stabilize the recombinant coronavirus S ectodomain trimer in the prefusion conformation.

6. The immunogen of claim 1, wherein the protomers of the S ectodomain trimer further comprise one or more mutations to a S 1/S2 protease cleavage site and/or a S2' protease cleavage site to inhibit protease cleavage.

7. The immunogen of claim 1, wherein the recombinant coronavirus S ectodomain trimer is soluble.

8. The immunogen of claim 1, wherein a C-terminal residue of the protomers in the ectodomain is linked to a transmembrane domain by a peptide linker, or is directly linked to the transmembrane domain.

9. The immunogen of claim 1, wherein a C-terminal residue of the S2 ectodomain is linked to a protein nanoparticle subunit by a peptide linker, or is directly linked to the protein nanoparticle subunit.

10. The immunogen of claim 9, wherein the protein nanoparticle subunit is a ferritin nanoparticle subunit.

11. A protein nanoparticle, comprising the immunogen of claim 9.

12. A virus-like particle comprising the immunogen of claim 1.

13. An isolated nucleic acid molecule encoding a protomer of the recombinant coronavirus S ectodomain trimer of claim 1.

14. The nucleic acid molecule of claim 13, operably linked to a promoter.

15. The nucleic acid molecule of claim 13, wherein the nucleic acid molecule is an RNA molecule.

16. A vector comprising the nucleic acid molecule of claim 13.

17. The vector of claim 16, wherein the vector is a viral vector.

18. An immunogenic composition comprising the immunogen of claim 1, and a pharmaceutically acceptable carrier.

19. A method of producing a recombinant coronavirus S ectodomain trimer stabilized in a prefusion conformation, comprising: expressing the nucleic acid molecule or vector of claim 13 in an isolated host cell to produce the recombinant coronavirus S ectodomain trimer; and purifying the recombinant coronavirus S ectodomain trimer.

20. The recombinant coronavirus S ectodomain trimer produced by the method of claim 19.

21. A method for generating an immune response to a coronavirus S ectodomain in a subject, comprising administering to the subject an effective amount of the immunogen of claim 1 to generate the immune response.

22. The method of claim 21, wherein the immune response treats or inhibits infection with the coronavirus.

23. The method of claim 21, wherein generating the immune response inhibits replication of the coronavirus in the subject.

Description

FIELD OF THE DISCLOSURE

This disclosure relates to recombinant coronavirus spike (S) proteins, such as Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV) S proteins, that are stabilized in a prefusion conformation by one or more amino acid substitutions, and their use as immunogens.

BACKGROUND

Coronaviruses are enveloped, positive-sense single-stranded RNA viruses. They have the largest genomes (26-32 kb) among known RNA viruses, and are phylogenetically divided into four genera (.alpha., .beta., .gamma., .delta.), with betacoronaviruses further subdivided into four lineages (A, B, C, D). Coronaviruses infect a wide range of avian and mammalian species, including humans. Of the six known human coronaviruses, four of them (HCoV-OC43, HCoV-229E, HCoV-HKU1 and HCoV-NL63) circulate annually in humans and generally cause mild respiratory diseases, although severity can be greater in infants, elderly, and the immunocompromised. In contrast, the Middle East respiratory syndrome coronavirus (MERS-CoV) and the severe acute respiratory syndrome coronavirus (SARS-CoV), belonging to betacoronavirus lineages C and B, respectively, are highly pathogenic. Both viruses emerged into the human population from animal reservoirs within the last 15 years and caused outbreaks with high case-fatality rates.

MERS-CoV was isolated in 2012 from a patient in Saudi Arabia and is still circulating across the Arabian Peninsula. Primary transmission, most likely from camels, is now considered to be the most common route of transmission, and camels are thought to be a secondary or intermediate reservoir for MERS-CoV, with bats serving as the primary reservoir. Human-to-human transmission, especially as a result of close contact between patients and hospital workers within health care settings, is another important route of transmission, and was responsible for an outbreak of MERS-CoV in South Korea. The high pathogenicity and airborne transmissibility of SARS-CoV and MERS-CoV have raised concern about the potential for another coronavirus pandemic. The high case-fatality rate, vaguely defined epidemiology, and absence of prophylactic or therapeutic measures against coronaviruses have created an urgent need for an effective vaccine and related therapeutic agents.

SUMMARY

Disclosed herein are recombinant coronavirus S ectodomain trimers comprising protomers comprising one or more proline substitution(s) that stabilize the S protein trimer in the prefusion conformation. One class of mutation, comprising one or more (such as two) proline substitutions at or near the boundary between a Heptad Repeat 1 (HR1) and a central helix of the protomers of the coronavirus S ectodomain trimer was found to be surprisingly effective for stabilization of coronavirus S protein trimers in the prefusion conformation. Embodiments of such prefusion-stabilized coronavirus S ectodomain trimers are demonstrated to produce a superior immune response in an animal model compared to corresponding coronavirus S ectodomain trimers that are not stabilized in the prefusion conformation.

In some embodiments, an immunogen is provided that comprises a recombinant alphacoronavirus or betacoronavirus S ectodomain trimer comprising protomers comprising one or two proline substitutions at or near a junction between a heptad repeat 1 (HR1) and a central helix that stabilize the S ectodomain trimer in a prefusion conformation. The one or two proline substitutions can comprise two consecutive proline substitutions (a "double proline substitution"). In some embodiments, the recombinant alphacoronavirus or betacoronavirus S ectodomain trimer comprises S ectodomains from a NL63-CoV, 229E-CoV, OC43-CoV, SARS-CoV, MERS-CoV, HKU1-CoV, WIV1-CoV, mouse hepatitis virus (MHV), or HKU9-CoV, that comprise the one or two proline substitutions.

In some embodiments, the recombinant alphacoronavirus or betacoronavirus S ectodomain trimer comprises: a recombinant HKU1-CoV S ectodomain trimer, and the double proline substitution is between residues 1050 to 1070 of the protomers in the trimer (for example, N1067P and L1068P substitutions); a recombinant SARS-CoV S ectodomain trimer, and the double proline substitution is between residues 951 to 971 of the protomers in the trimer (for example, K968P and V969P substitutions); a recombinant MERS-CoV S ectodomain trimer, and the double proline substitution is between residues 1050 to 1069 of the protomers in the trimer (for example, V1060P and L1061P substitutions); a recombinant OC43-CoV S ectodomain trimer, and the double proline substitution is between residues 1062 to 1082 of the protomers in the trimer (for example, A1079P and L1080P substitutions); a recombinant HKU9-CoV S ectodomain trimer, and the double proline substitution is between residues 966 to 986 of the protomers in the trimer (for example, G1018P and L1019P substitutions); a recombinant NL63-CoV S ectodomain trimer, and the double proline substitution is between residues 1035 to 1055 of the protomers in the trimer (for example, S1052P and I1053P substitutions); a recombinant 229E-CoV S ectodomain trimer, and the double proline substitution is between residues 852 to 872 of the protomers in the trimer (for example, I869P and I870P substitutions); a recombinant WIV1-CoV S ectodomain trimer, and the double proline substitution is between residues 952 to 972 of the protomers in the trimer (for example, K969P and V970P substitutions); or a recombinant MHV S ectodomain trimer, and the double proline substitution is between residues 852 to 872 of the protomers in the trimer (for example, I869P and I870P substitutions).

In some embodiments, the protomers of the recombinant alphacoronavirus or betacoronavirus S ectodomain trimer further comprise one or more additional amino acid substitutions or deletions, such as amino acid substitutions that stabilize the recombinant

alphacoronavirus or betacoronavirus S ectodomain trimer in the prefusion conformation, or amino acid substitutions to inhibit or prevent protease cleavage at a S1/S2 protease cleavage site and/or a S2' protease cleave site of the S ectodomain.

In some embodiments, the protomers of the recombinant alphacoronavirus or betacoronavirus S ectodomain trimer can be linked to a trimerization domain (such as T4 Fibritin trimerization domain). In additional embodiments, the protomers of the recombinant alphacoronavirus or betacoronavirus S ectodomain trimer can be linked to a transmembrane domain.

In additional embodiments, the recombinant coronavirus S ectodomain trimer can be included on a protein nanoparticle, such as a ferritin protein nanoparticle. Nucleic acid molecules encoding a protomer of the disclosed recombinant coronavirus S ectodomain trimers are also provided, as are vectors including the nucleic acid molecules, and methods of producing the disclosed coronavirus S ectodomain trimers.

Immunogenic compositions including the recombinant coronavirus S ectodomain trimer that are suitable for administration to a subject are also provided, and may also be contained in a unit dosage form. The compositions can further include an adjuvant. The recombinant coronavirus S ectodomain trimers may also be conjugated to a carrier to facilitate presentation to the immune system.

Methods of inducing an immune response in a subject are disclosed, as are methods of treating, inhibiting or preventing a coronavirus infection in a subject, by administering to the subject an effective amount of a disclosed recombinant coronavirus S ectodomain trimer, nucleic acid molecule, or vector.

The foregoing and other features and advantages of this disclosure will become more apparent from the following detailed description of several embodiments which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

FIGS. 1A-1C illustrate the structure of the HKU1-CoV prefusion spike ectodomain. (1A) A single protomer of the trimeric S protein is shown in cartoon representation colored as a rainbow from the N to C terminus (blue to red) with the reconstructed EM density of remaining protomers shown in white and grey. (1B) The S1 subunit is composed of the N-terminal domain (NTD) and C-terminal domain (CTD) as well as two sub-domains (SD-1 and SD-2). The S2 subunit contains the coronavirus fusion machinery and is primarily .alpha.-helical. (1C) Domain architecture of the HKU1-CoV S protein colored as in (1A).

FIGS. 2A-2D illustrate the architecture of the HKU1-CoV S1 subunit. (2A) EM density corresponding to each S1 protomer is shown. The putative glycan-binding and protein-receptor-binding sites are indicated with dashed shapes on the NTD and CTD, respectively. (2B) The HKU1-CoV S1 CTD forms quaternary interactions with an adjacent CTD using a surface similar to that used by SARS-CoV CTD to bind its receptor, ACE2. (2C) SD-1 is composed of amino acid residues before and after the S1 CTD. (2D) SD-2 is composed of S1 sequence C-terminal to the CTD, a short peptide following the NTD, and the N-terminal strand of S2, which follows the S1/S2 furin-cleavage site.

FIGS. 3A-3C illustrate the HKU1-CoV S2 subunit fusion machinery. (3A) The HKU1-CoV S2 subunit is colored like a rainbow from the N-terminal .beta.-strand (blue), which participates in S1 sub-domain 2, to the C terminus (red) before HR2. (3B) The HKU1-CoV S2 structure contains the fusion peptide (FP) and a HR1. Protease-recognition sites are indicated within disordered regions of the protein (dashed lines). (3C) A comparison of coronavirus S2 HR1 in the pre- and post-fusion conformations. Five HR1 .alpha.-helices are labelled and colored like a rainbow from blue to red, N to C terminus, respectively. The structures are oriented to position similar portions of the central helix (red).

FIGS. 4A-4C illustrate stabilization of MERS-CoV S protein in a prefusion conformation by V1060P ("Top 3") and L1061P ("Top 4") amino acid substitutions. (4A) Location of various stabilization design conceptions. V1060P and L1061P (red circle) are located at the top of S2 HR1 and the S2 central helix. MERS-CoV S ectodomains with V1060P and L1061P mutations were expressed individually and in combination and purified. Protein expression levels and purity were determined by (4B) gel electrophoresis and (4C) size-exclusion chromatography.

FIGS. 5A-5B are a set of graphs showing results from neutralization assays using sera from mice immunized with the MERS-CoV S prefusion stabilized (2P) ectodomain trimer. Mice (N=5/group) were immunized with 0.1 .mu.g of MERS-CoV wild-type S ectodomain trimer or MERS-CoV prefusion-stabilized S ectodomain trimer intramuscularly with Sigma Adjuvant System at weeks 0 and 3. Control mice were given PBS. Two weeks following the last immunization, serum was collected and tested for neutralizing antibodies against various MERS pseudovirus strains: England1, Florida USA2, Bisha1, Korea002, JordanN3, Buraidah1, and Indiana USA1. (FIG. 5A) Reciprocal serum IC.sub.90 neutralizing activity against autologous MERS England1 pseudotyped lentivirus reporter plotted against vaccine dose. (FIG. 5B) Reciprocal serum IC.sub.90 neutralizing activity against multiple homologous MERS-CoV pseudoviruses of sera from mice immunized with 0.1 .mu.g of purified MERS-CoV S ectodomain trimer. For both panels, the

geometric mean IC.sub.90 titer (GMT) of each group is represented by (FIG. 5A) symbols or (FIG. 5B) bars. Error bars represent geometric SDs. P values denoted as *P<0.05 and **P<0.01. The limit of detection for the assay is represented by dotted lines; for sera below the limit of detection a reciprocal IC.sub.90 titer of 10 was assigned.

FIGS. 6A and 6B shows results from the dissection of binding and neutralizing antibodies elicited by MERS S-2P. Serum from mice immunized with (A) MERS 51, (B) MERS S WT ectodomain trimer, and MERS S-2P ectodomain trimer were depleted of MERS RBD, MERS 51, and MERS S-2P ectodomain trimer specific antibodies by magnetic bead depletion. The resulting depleted serum was then tested for (FIG. 6A) MERS S-2P ectodomain trimer specific antibodies by ELISA or (FIG. 6B) neutralizing antibodies against MERS England1 pseudovirus. For the binding assays. endpoint ELISA titers were determined, and % binding retained was calculated as a measure of endpoint titers for each serum depleted with MERS protein compared to binding after depletion with a nonspecific protein. For the neutralization assays, IC50 titers were determined, and % neutralization retained was calculated as a measure of neutralization each serum depleted with MERS protein compared to binding after depletion with a nonspecific protein. Bars represent the mean of each group; error bars represent SD.

FIG. 7 is a set of graphs showing that MERS-CoV S-2P immunization protects against lethal MERS challenge in mice. C57BL/6J mice were genetically engineered using CRISPR-Cas9 genomic editing to encode human DPP4 mutations (288L and 330R; "288/330.sup.+/+") as previously described (see, Cockrell et al., "A mouse model for MERS coronavirus-induced acute respiratory distress syndrome." Nature Microbiology. 2:16226, 2016, which is incorporated by reference herein). 288/330.sup.+/+ mice were vaccinated with 0.1 .mu.g MERS-CoV S-2P or PBS, with Sigma Adjuvant System at weeks 0 and 3. Four weeks following final vaccination, mice were challenged with a lethal dose of mouse-adapted MERS virus and monitored for survival and weight loss.

FIG. 8 illustrates the structural domains of the HKU1-CoV, SARS-CoV, and MERS-CoV S proteins, as well as positioning of double proline substitutions to stabilize these proteins in the prefusion conformation.

FIGS. 9A-9C show a sequence alignment of the S2 subunit of the HKU1-CoV (SEQ ID NO: 8), SARS-CoV (SEQ ID NO: 6), MERS-CoV (SEQ ID NO: 1), HKU9-CoV (SEQ ID NO: 12), NL63-CoV (SEQ ID NO: 18), and 229E-CoV (SEQ ID NO: 20) S proteins, showing relevant sequence homology.

FIG. 10 shows a Coomassie-stained polyacrylamide gel illustrating that introduction of proline substitutions in the SARS-CoV (K968P and V969P substitutions, SARS-S-2P) and HKU1-CoV (N1067P and L1068P substitutions, HKU1-S-2P) S ectodomains at the locations corresponding to the MERS-CoV S V1060P and L1061P substitutions boosts the expression of the SARS-CoV and HKU1-CoV S ectodomains.

FIG. 11 shows a Coomassie-stained polyacrylamide gel illustrating that the SARS-CoV S ectodomain with K968P and V969P substitutions (SARS-S-2P) has higher thermal stability than corresponding SARS-CoV S ectodomain having native sequence (SARS-S-WT).

FIG. 12 shows a set of graphs illustrating gel chromatography results of purified SARS-CoV, MERS-CoV, and HKU1-CoV S ectodomains having native (S-WT) sequence or double proline substitutions noted above (S-2P).

FIGS. 13A-13C show images of negative-stain electron microscopy of purified ectodomain trimers of MERS-CoV S 2P (V1060P and L1061P, SEQ ID NO: 28), SARS-CoV S 2P (K968P and V969P, SEQ ID NO: 30), HKU1-CoV S 2P (N1067P and L1068P, SEQ ID NO: 31), OC43-CoV S 2P (A1079P and L1080P, SEQ ID NO: 33), WIV1-CoV S 2P (K969P and V970P, SEQ ID NO: 34), PEDV-CoV S 2P (I1076P and L1077P, SEQ ID NO: 40), 229E S-2P (I869P and 1870P, SEQ ID NO: 37), and SDCV 2-2P. Each of these ectodomain trimers was purified as a single peak and formed trimers in the typical prefusion conformation.

FIGS. 14A-14G show low-resolution negative-stain reconstructions of S trimer constructs from (14A) HKU1-CoV S 2P ectodomain trimer, (14B) MERS-CoV S 2P ectodomain trimer, (14C) SARS-CoV S 2P ectodomain trimer, (14D) OC43 S-2P ectodomain trimer, (14E) WIV1-CoV S 2P ectodomain trimer, (14F) PEDV-CoV S 2P ectodomain trimer, and (14G) 229E-CoV S 2P ectodomain trimer that were obtained from the negative-stain electron microscopy data shown in FIG. 13. The particles all formed homogeneous trimeric spike protein structures.

FIG. 15 is a graph showing results of immunogenicity assays of HKU1-CoV S 2P ectodomain trimer and SARS S-2P ectodomain trimer in mice. Reciprocal serum IC.sub.90 neutralizing activity against autologous pseudotyped lentivirus reporter (SARS Urbani for the SARS immunization) plotted against vaccine dose. The geometric mean IC.sub.90 titer (GMT) of each group is represented by symbols. Error bars represent geometric SDs. The limit of detection for the assay is represented by dotted lines; for sera below the limit of detection a reciprocal IC.sub.90 titer of 10 was assigned.

FIG. 16 shows results from immunogenicity assays in mice using the OC43-CoV S-2P and WIV1-CoV S-2P ectodomain trimer immunogens. BALB/c mice were vaccinated with 1 .mu.g of OC43 S-2P ectodomain trimer or WIV1-CoV S-2P ectodomain trimer, with Sigma Adjuvant System at weeks 0 and 3. Two weeks following final vaccination, mice were bled for antibody analysis. Binding antibody titers to OC43 S-2P ectodomain trimer or WIV1-CoV S-2P ectodomain trimer were measured by ELISA. The geometric

mean titer (GMT) and geometric SDs of each group are represented. The dotted line represents the assay limit of detection. ** denotes p-value <0.01, determined by Mann-Whitney t-test.

SEQUENCE LISTING

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. The Sequence Listing is submitted as an ASCII text file in the form of the file named "Sequence.txt" (.about.404 kb), which was created on Apr. 19, 2019 and which is incorporated by reference herein.

DETAILED DESCRIPTION

Past efforts to develop coronavirus vaccines have used whole-inactivated virus, live-attenuated virus, recombinant protein subunit, or genetic approaches (Graham et al., Nature reviews. Microbiology 11, 836, 2013). This disclosure provides CoV Spike glycoprotein (S) ectodomain trimers that are stabilized in the prefusion conformation and which are shown to elicit a neutralizing immune response in animal models.

I. Summary of Terms

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, Genes X, published by Jones & Bartlett Publishers, 2009; and Meyers et al. (eds.), The Encyclopedia of Cell Biology and Molecular Medicine, published by Wiley-VCH in 16 volumes, 2008; and other similar references.

As used herein, the singular forms "a," "an," and "the," refer to both the singular as well as plural, unless the context clearly indicates otherwise. For example, the term "an antigen" includes single or plural antigens and can be considered equivalent to the phrase "at least one antigen." As used herein, the term "comprises" means "includes." It is further to be understood that any and all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for descriptive purposes, unless otherwise indicated. Although many methods and materials similar or equivalent to those described herein can be used, particular suitable methods and materials are described herein. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting. To facilitate review of the various embodiments, the following explanations of terms are provided:

Adjuvant: A vehicle used to enhance antigenicity. In some embodiments, an adjuvant can include a suspension of minerals (alum, aluminum hydroxide, or phosphate) on which antigen is adsorbed; or water-in-oil emulsion, for example, in which antigen solution is emulsified in mineral oil (Freund incomplete adjuvant), sometimes with the inclusion of killed mycobacteria (Freund's complete adjuvant) to further enhance antigenicity (inhibits degradation of antigen and/or causes influx of macrophages). In some embodiments, the adjuvant used in a disclosed immunogenic composition is a combination of lecithin and carbomer homopolymer (such as the ADJUPLEX.TM. adjuvant available from Advanced BioAdjuvants, LLC, see also Wegmann, Clin Vaccine Immunol, 22(9): 1004-1012, 2015). Additional adjuvants for use in the disclosed immunogenic compositions include the QS21 purified plant extract, Matrix M, ASO1, MF59, and ALFQ adjuvants. Immunostimulatory oligonucleotides (such as those including a CpG motif) can also be used as adjuvants. Adjuvants include biological molecules (a "biological adjuvant"), such as costimulatory molecules. Exemplary adjuvants include IL-2, RANTES, GM-CSF, TNF-.alpha., IFN-.gamma., G-CSF, LFA-3, CD72, B7-1, B7-2, OX-40L, 4-1BBL and toll-like receptor (TLR) agonists, such as TLR-9 agonists. Additional description of adjuvants can be found, for example, in Singh (ed.) Vaccine Adjuvants and Delivery Systems. Wiley-Interscience, 2007). Adjuvants can be used in combination with the disclosed immunogens.

Administration: The introduction of an agent, such as a disclosed immunogen, into a subject by a chosen route. Administration can be local or systemic. For example, if the chosen route is intranasal, the agent (such as an immunogen comprising a recombinant coronavirus S ectodomain trimer stabilized in a prefusion conformation) is administered by introducing the composition into the nasal passages of the subject. Exemplary routes of administration include, but are not limited to, oral, injection (such as subcutaneous, intramuscular, intradermal, intraperitoneal, and intravenous), sublingual, rectal, transdermal (for example, topical), intranasal, vaginal, and inhalation routes.

Amino acid substitution: The replacement of one amino acid in a polypeptide with a different amino acid.

Antibody: An immunoglobulin, antigen-binding fragment, or derivative thereof, that specifically binds and recognizes an analyte (antigen) such as a coronavirus S protein, an antigenic fragment thereof, or a dimer or multimer of the antigen. The term "antibody" is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments, so long as they exhibit the desired antigen-binding activity. Non-limiting examples of antibodies include, for example, intact immunoglobulins and variants and fragments thereof that retain binding affinity for the antigen. Examples of antibody fragments include but are not limited to Fv, Fab,

Fab', Fab'-SH, F(ab').sub.2; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments. Antibody fragments include antigen binding fragments either produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA methodologies (see, e.g., Kontermann and Dubel (Ed), Antibody Engineering, Vols. 1-2, 2.sup.nd Ed., Springer Press, 2010).

Carrier: An immunogenic molecule to which an antigen can be linked. When linked to a carrier, the antigen may become more immunogenic. Carriers are chosen to increase the immunogenicity of the antigen and/or to elicit antibodies against the carrier which are diagnostically, analytically, and/or therapeutically beneficial. Useful carriers include polymeric carriers, which can be natural (for example, proteins from bacteria or viruses), semi-synthetic or synthetic materials containing one or more functional groups to which a reactant moiety can be attached.

Cavity-filling amino acid substitution: An amino acid substitution that fills a cavity within the protein core of a protein, such as a coronavirus S protein ectodomain. Cavities are essentially voids within a folded protein where amino acids or amino acid side chains are not present. In several embodiments, a cavity-filling amino acid substitution is introduced to fill a cavity present in the prefusion conformation of a coronavirus S ectodomain core that collapses (e.g., has reduced volume) after transition to the postfusion conformation.

Conservative variants: "Conservative" amino acid substitutions are those substitutions that do not substantially affect or decrease a function of a protein, such as the ability of the protein to induce an immune response when administered to a subject. The term conservative variation also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid. Furthermore, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (for instance less than 5%, in some embodiments less than 1%) in an encoded sequence are conservative variations where the alterations result in the substitution of an amino acid with a chemically similar amino acid.

The following six groups are examples of amino acids that are considered to be conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);

2) Aspartic acid (D), Glutamic acid (E);

3) Asparagine (N), Glutamine (Q);

4) Arginine (R), Lysine (K);

5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and

6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

Non-conservative substitutions are those that reduce an activity or function of the recombinant coronavirus S ectodomain trimer, such as the ability to induce an immune response when administered to a subject. For instance, if an amino acid residue is essential for a function of the protein, even an otherwise conservative substitution may disrupt that activity. Thus, a conservative substitution does not alter the basic function of a protein of interest.

Control: A reference standard. In some embodiments, the control is a negative control sample obtained from a healthy patient. In other embodiments, the control is a positive control sample obtained from a patient diagnosed with a coronavirus infection, such as MERS-CoV or SARS-CoV. In still other embodiments, the control is a historical control or standard reference value or range of values (such as a previously tested control sample, such as a group of patients infected with a coronavirus with known prognosis or outcome, or group of samples that represent baseline or normal values).

A difference between a test sample and a control can be an increase or conversely a decrease. The difference can be a qualitative difference or a quantitative difference, for example a statistically significant difference. In some examples, a difference is an increase or decrease, relative to a control, of at least about 5%, such as at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 150%, at least about 200%, at least about 250%, at least about 300%, at least about 350%, at least about 400%, at least about 500%, or greater than 500%.

Coronavirus: A family of positive-sense, single-stranded RNA viruses that are known to cause severe respiratory illness. Viruses currently known to infect human from the coronavirus family are from the alphacoronavirus and betacoronavirus genera. Additionally, it is believed that the gammacoronavirus and deltacoronavirus genera may infect humans in the future.

Non-limiting examples of betacoronaviruses include Middle East respiratory syndrome coronavirus (MERS-CoV), Severe Acute Respiratory Syndrome coronavirus (SARS-CoV), Human coronavirus HKU1 (HKU1-CoV), Human coronavirus OC43 (OC43-CoV),

Murine Hepatitis Virus (MHV-CoV), Bat SARS-like coronavirus WIV1 (WIV1-CoV), and Human coronavirus HKU9 (HKU9-CoV). Non-limiting examples of alphacoronaviruses include human coronavirus 229E (229E-CoV), human coronavirus NL63 (NL63-CoV), porcine epidemic diarrhea virus (PEDV), and Transmissible gastroenteritis coronavirus (TGEV). A non-limiting example of a deltacoronaviruses is the Swine Delta Coronavirus (SDCV). Exemplary sequences of the ectodomains of S proteins from these viruses are provided herein.

The viral genome is capped, polyadenylated, and covered with nucleocapsid proteins. The coronavirus virion includes a viral envelope containing type I fusion glycoproteins referred to as the spike (S) protein. Most coronaviruses have a common genome organization with the replicase gene included in the 5'-portion of the genome, and structural genes included in the 3'-portion of the genome.

Coronavirus Spike (S) protein: A class I fusion glycoprotein initially synthesized as a precursor protein. Individual precursor S polypeptides form a homotrimer and undergo glycosylation within the Golgi apparatus as well as processing to remove the signal peptide, and cleavage by a cellular protease to generate separate 51 and S2 polypeptide chains, which remain associated as S1/S2 protomers within the homotrimer and is therefore a trimer of heterodimers. The 51 subunit is distal to the virus membrane and contains the receptor-binding domain (RBD) that mediates virus attachment to its host receptor. The S2 subunit contains fusion protein machinery, such as the fusion peptide, two heptad-repeat sequences (HR1 and HR2) and a central helix typical of fusion glycoproteins, a transmembrane domain, and the cytosolic tail domain.

Coronavirus Spike (S) protein prefusion conformation: A structural conformation adopted by the ectodomain of the coronavirus S protein following processing into a mature coronavirus S protein in the secretory system, and prior to triggering of the fusogenic event that leads to transition of coronavirus S to the postfusion conformation. The three-dimensional structure of an exemplary coronavirus S protein (HKU1-CoV) in a prefusion conformation is disclosed herein (see Example 1) and provided in Kirchdoerfer et al., "Pre-fusion structure of a human coronavirus spike protein," Nature, 531: 118-121, 2016 (incorporated by reference herein).

A coronavirus S ectodomain trimer "stabilized in a prefusion conformation" comprises one or more amino acid substitutions, deletions, or insertions compared to a native coronavirus S sequence that provide for increased retention of the prefusion conformation compared to coronavirus S ectodomain trimers formed from a corresponding native coronavirus S sequence. The "stabilization" of the prefusion conformation by the one or more amino acid substitutions, deletions, or insertions can be, for example, energetic stabilization (for example, reducing the energy of the prefusion conformation relative to the post-fusion open conformation) and/or kinetic stabilization (for example, reducing the rate of transition from the prefusion conformation to the postfusion conformation). Additionally, stabilization of the coronavirus S ectodomain trimer in the prefusion conformation can include an increase in resistance to denaturation compared to a corresponding native coronavirus S sequence. Methods of determining if a coronavirus S ectodomain trimer is in the prefusion conformation are provided herein, and include (but are not limited to) negative-stain electron microscopy and antibody binding assays using a prefusion-conformation-specific antibody.

Degenerate variant: In the context of the present disclosure, a "degenerate variant" refers to a polynucleotide encoding a polypeptide that includes a sequence that is degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences encoding a peptide are included as long as the amino acid sequence of the peptide encoded by the nucleotide sequence is unchanged.

Effective amount: An amount of agent, such as an immunogen, that is sufficient to elicit a desired response, such as an immune response in a subject. It is understood that to obtain a protective immune response against an antigen of interest can require multiple administrations of a disclosed immunogen, and/or administration of a disclosed immunogen as the "prime" in a prime boost protocol wherein the boost immunogen can be different from the prime immunogen. Accordingly, an effective amount of a disclosed immunogen can be the amount of the immunogen sufficient to elicit a priming immune response in a subject that can be subsequently boosted with the same or a different immunogen to elicit a protective immune response.

In one example, a desired response is to inhibit or reduce or prevent CoV (such as MERS-CoV) infection. The CoV infection does not need to be completely eliminated or reduced or prevented for the method to be effective. For example, administration of an effective amount of the immunogen can induce an immune response that decreases the CoV infection (for example, as measured by infection of cells, or by number or percentage of subjects infected by the CoV) by a desired amount, for example by at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even at least 100% (elimination or prevention of detectable CoV infection), as compared to a suitable control.

Epitope: An antigenic determinant. These are particular chemical groups or peptide sequences on a molecule that are antigenic, such that they elicit a specific immune response, for example, an epitope is the region of an antigen to which B and/or T cells respond. An antibody can bind to a particular antigenic epitope, such as an epitope on coronavirus S ectodomain, such as a MERS-CoV S ectodomain. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein.

Expression: Transcription or translation of a nucleic acid sequence. For example, a gene is expressed when its DNA is transcribed into an RNA or RNA fragment, which in some examples is processed to become mRNA. A gene may also be expressed when its mRNA is

translated into an amino acid sequence, such as a protein or a protein fragment. In a particular example, a heterologous gene is expressed when it is transcribed into an RNA. In another example, a heterologous gene is expressed when its RNA is translated into an amino acid sequence. The term "expression" is used herein to denote either transcription or translation. Regulation of expression can include controls on transcription, translation, RNA transport and processing, degradation of intermediary molecules such as mRNA, or through activation, inactivation, compartmentalization or degradation of specific protein molecules after they are produced.

Expression Control Sequences: Nucleic acid sequences that regulate the expression of a heterologous nucleic acid sequence to which it is operatively linked. Expression control sequences are operatively linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus expression control sequences can include appropriate promoters, enhancers, transcription terminators, a start codon (ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The term "control sequences" is intended to include, at a minimum, components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Expression control sequences can include a promoter.

A promoter is a minimal sequence sufficient to direct transcription. Also included are those promoter elements which are sufficient to render promoter-dependent gene expression controllable for cell-type specific, tissue-specific, or inducible by external signals or agents; such elements may be located in the 5' or 3' regions of the gene. Both constitutive and inducible promoters are included (see for example, Bitter et al., Methods in Enzymology 153:516-544, 1987). For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage lambda, plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used. In one embodiment, when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (such as metallothionein promoter) or from mammalian viruses (such as the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) can be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the nucleic acid sequences.

Expression vector: A vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (e.g., naked or contained in liposomes) and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

Glycosylation site: An amino acid sequence on the surface of a polypeptide, such as a protein, which accommodates the attachment of a glycan. An N-linked glycosylation site is triplet sequence of NX(S/T) in which N is asparagine, X is any residues except proline, and (S/T) is a serine or threonine residue. A glycan is a polysaccharide or oligosaccharide. Glycan may also be used to refer to the carbohydrate portion of a glycoconjugate, such as a glycoprotein, glycolipid, or a proteoglycan.

Heterologous: Originating from a different genetic source. A nucleic acid molecule that is heterologous to a cell originated from a genetic source other than the cell in which it is expressed. In one specific, non-limiting example, a heterologous nucleic acid molecule encoding a recombinant coronavirus S ectodomain is expressed in a cell, such as a mammalian cell. Methods for introducing a heterologous nucleic acid molecule in a cell or organism are well known in the art, for example transformation with a nucleic acid, including electroporation, lipofection, particle gun acceleration, and homologous recombination.

Ferritin: A protein that stores iron and releases it in a controlled fashion. The protein is produced by almost all living organisms. Ferritin polypeptides assemble into a globular protein complex of 24 protein subunits, and each of the 24 subunits includes a single ferritin polypeptide. In some examples, ferritin is used to form a nanoparticle presenting antigens on its surface, for example, a coronavirus S ectodomain trimer.

Host cells: Cells in which a vector can be propagated and its DNA expressed. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used.

Immune response: A response of a cell of the immune system, such as a B cell, T cell, or monocyte, to a stimulus. In one embodiment, the response is specific for a particular antigen (an "antigen-specific response"). In one embodiment, an immune response is a T cell response, such as a CD4+ response or a CD8+ response. In another embodiment, the response is a B cell response, and results in the production of specific antibodies.

Immunogen: A compound, composition, or substance (for example, a recombinant coronavirus S ectodomain trimer) that can elicit an immune response in an animal, including compositions that are injected or absorbed into an animal. Administration of an immunogen to a subject can lead to protective immunity against a pathogen of interest.

Immunogenic composition: A composition comprising a disclosed recombinant coronavirus S ectodomain trimer that induces a measurable CTL response against the coronavirus, or induces a measurable B cell response (such as production of antibodies) against

the coronavirus, when administered to a subject. It further refers to isolated nucleic acid molecules and vectors encoding a protomer of a disclosed recombinant coronavirus S ectodomain trimer that can be used to express the protomer (and thus be used to elicit an immune response against recombinant coronavirus S ectodomain trimer). For in vivo use, the immunogenic composition will typically include the recombinant coronavirus S ectodomain trimer or a nucleic acid molecule encoding a protomer of the recombinant coronavirus S ectodomain trimer in a pharmaceutically acceptable carrier and may also include other agents, such as an adjuvant.

Inhibiting or treating a disease: Inhibiting the full development of a disease or condition, for example, in a subject who is at risk for a disease such as a CoV infection. "Treatment" refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop. The term "ameliorating," with reference to a disease or pathological condition, refers to any observable beneficial effect of the treatment. Inhibiting a disease can include preventing or reducing the risk of the disease, such as preventing or reducing the risk of viral infection. The beneficial effect can be evidenced, for example, by a delayed onset of clinical symptoms of the disease in a susceptible subject, a reduction in severity of some or all clinical symptoms of the disease, a slower progression of the disease, a reduction in the viral load, an improvement in the overall health or well-being of the subject, or by other parameters that are specific to the particular disease. A "prophylactic" treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs for the purpose of decreasing the risk of developing pathology.

Isolated: An "isolated" biological component has been substantially separated or purified away from other biological components, such as other biological components in which the component naturally occurs, such as other chromosomal and extrachromosomal DNA, RNA, and proteins. Proteins, peptides, nucleic acids, and viruses that have been "isolated" include those purified by standard purification methods.

Isolated does not require absolute purity, and can include protein, peptide, nucleic acid, or virus molecules that are at least 50% isolated, such as at least 75%, 80%, 90%, 95%, 98%, 99%, or even 99.9% isolated.

Linker and Linked: A bi-functional molecule that can be used to link two molecules into one contiguous molecule. Non-limiting examples of peptide linkers include glycine-serine peptide linkers. Unless context indicates otherwise, reference to "linking" a first polypeptide and a second polypeptide, or to two polypeptides "linked" together, or to a first polypeptide having a "linkage" to a second polypeptide, refers to covalent linkage by peptide bond (for example via a peptide linker) such that the first and second polypeptides form a contiguous polypeptide chain. If a peptide linker is involved, the covalent linkage of the first and second polypeptides can be to the N- and C-termini of the peptide linker Typically, such linkage is accomplished using molecular biology techniques to genetically manipulate DNA encoding the first polypeptide linked to the second polypeptide by the peptide linker.

Native protein, sequence, or disulfide bond: A polypeptide, sequence or disulfide bond that has not been modified, for example, by selective mutation. For example, selective mutation to focus the antigenicity of the antigen to a target epitope, or to introduce a disulfide bond into a protein that does not occur in the native protein. Native protein or native sequence are also referred to as wild-type protein or wild-type sequence. A non-native disulfide bond is a disulfide bond that is not present in a native protein, for example, a disulfide bond that forms in a protein due to introduction of one or more cysteine residues into the protein by genetic engineering.

Nucleic acid molecule: A polymeric form of nucleotides, which may include both sense and anti-sense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed polymers of the above. A nucleotide refers to a ribonucleotide, deoxynucleotide or a modified form of either type of nucleotide. The term "nucleic acid molecule" as used herein is synonymous with "nucleic acid" and "polynucleotide." A nucleic acid molecule is usually at least 10 bases in length, unless otherwise specified. The term includes single- and double-stranded forms of DNA. A polynucleotide may include either or both naturally occurring and modified nucleotides linked together by naturally occurring and/or non-naturally occurring nucleotide linkages. "cDNA" refers to a DNA that is complementary or identical to an mRNA, in either single stranded or double stranded form. "Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked nucleic acid sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

Pharmaceutically acceptable carriers: The pharmaceutically acceptable carriers of use are conventional. Remington's Pharmaceutical Sciences, by E. W. Martin, Mack Publishing Co., Easton, Pa., 19th Edition, 1995, describes compositions and formulations suitable for pharmaceutical delivery of the disclosed immunogens.

In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (e.g., powder,

pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral carriers, pharmaceutical compositions (such as immunogenic compositions) to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate. In particular embodiments, suitable for administration to a subject the carrier may be sterile, and/or suspended or otherwise contained in a unit dosage form containing one or more measured doses of the composition suitable to induce the desired immune response. It may also be accompanied by medications for its use for treatment purposes. The unit dosage form may be, for example, in a sealed vial that contains sterile contents or a syringe for injection into a subject, or lyophilized for subsequent solubilization and administration or in a solid or controlled release dosage.

Polypeptide: Any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation). "Polypeptide" applies to amino acid polymers including naturally occurring amino acid polymers and non-naturally occurring amino acid polymer as well as in which one or more amino acid residue is a non-natural amino acid, for example, an artificial chemical mimetic of a corresponding naturally occurring amino acid. A "residue" refers to an amino acid or amino acid mimetic incorporated in a polypeptide by an amide bond or amide bond mimetic. A polypeptide has an amino terminal (N-terminal) end and a carboxy terminal (C-terminal) end. "Polypeptide" is used interchangeably with peptide or protein, and is used herein to refer to a polymer of amino acid residues.

Prime-boost vaccination: An immunotherapy including administration of a first immunogenic composition (the primary vaccine) followed by administration of a second immunogenic composition (the booster vaccine) to a subject to induce an immune response. The priming vaccine and/or the booster vaccine include a vector (such as a viral vector, RNA, or DNA vector) expressing the antigen to which the immune response is directed. The booster vaccine is administered to the subject after the priming vaccine; a suitable time interval between administration of the priming vaccine and the booster vaccine, and examples of such timeframes are disclosed herein. In some embodiments, the priming vaccine, the booster vaccine, or both primer vaccine and the booster vaccine additionally include an adjuvant. In one non-limiting example, the priming vaccine is a DNA-based vaccine (or other vaccine based on gene delivery), and the booster vaccine is a protein subunit or protein nanoparticle based vaccine.

Protein nanoparticle: A multi-subunit, self-assembling, protein-based polyhedron shaped structure. The subunits are each composed of proteins (for example a glycosylated polypeptide), and, optionally of single or multiple features of the following: nucleic acids, prosthetic groups, organic and inorganic compounds. In some embodiments, protomers of the disclosed trimeric spike proteins can be fused to the subunits of the protein nanoparticles to provide multiple copies of the trimeric spike on each protein nanoparticle. Non-limiting examples of protein nanoparticles include ferritin nanoparticles (see, e.g., Zhang, Y. Int. J. Mol. Sci., 12:5406-5421, 2011, incorporated by reference herein), encapsulin nanoparticles (see, e.g., Sutter et al., Nature Struct. and Mol. Biol., 15:939-947, 2008, incorporated by reference herein), Sulfur Oxygenase Reductase (SOR) nanoparticles (see, e.g., Urich et al., Science, 311:996-1000, 2006, incorporated by reference herein), lumazine synthase nanoparticles (see, e.g., Zhang et al., J. Mol. Biol., 306: 1099-1114, 2001), and pyruvate dehydrogenase nanoparticles (see, e.g., Izard et al., PNAS 96: 1240-1245, 1999, incorporated by reference herein). Ferritin, encapsulin, SOR, lumazine synthase, and pyruvate dehydrogenase are monomeric proteins that self-assemble into a globular protein complexes that in some cases consists of 24, 60, 24, 60, and 60 protein subunits, respectively. Additional protein nanoparticle structures are described by Heinze et al., J Phys Chem B., 120(26):5945-52, 2016; Hsia et al., Nature, 535(7610):136-9, 2016; and King et al., Nature, 510(7503):103-8, 2014; each of which is incorporated by reference herein.

Recombinant: A recombinant nucleic acid molecule is one that has a sequence that is not naturally occurring, for example, includes one or more nucleic acid substitutions, deletions or insertions, and/or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination can be accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, for example, by genetic engineering techniques. A recombinant virus is one that includes a genome that includes a recombinant nucleic acid molecule. A recombinant protein is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. In several embodiments, a recombinant protein is encoded by a heterologous (for example, recombinant) nucleic acid that has been introduced into a host cell, such as a bacterial or eukaryotic cell, or into the genome of a recombinant virus.

Sequence identity: The similarity between amino acid sequences is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity; the higher the percentage, the more similar the two sequences are. Homologs, orthologs, or variants of a polypeptide will possess a relatively high degree of sequence identity when aligned using standard methods.

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith & Waterman, Adv. Appl. Math. 2:482, 1981; Needleman & Wunsch, J. Mol. Biol. 48:443, 1970; Pearson & Lipman, Proc. Natl. Acad. Sci. USA 85:2444, 1988; Higgins & Sharp, Gene, 73:237-44, 1988; Higgins & Sharp, CABIOS 5:151-3, 1989; Corpet et al., Nuc. Acids Res. 16:10881-90, 1988; Huang et al. Computer Appls. in the Biosciences 8, 155-65, 1992; and Pearson et al., Meth. Mol. Bio. 24:307-31, 1994. Altschul et al., J. Mol. Biol. 215:403-10, 1990, presents a detailed consideration of sequence alignment methods and homology calculations.

Homologs and variants of a polypeptide (such as a coronavirus S ectodomain) are typically characterized by possession of at least about 75%, for example at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity counted over the full length alignment with the amino acid sequence of interest. Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity. When less than the entire sequence is being compared for sequence identity, homologs and variants will typically possess at least 80% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85% or at least 90% or 95% depending on their similarity to the reference sequence. Methods for determining sequence identity over such short windows are available at the NCBI website on the internet.

As used herein, reference to "at least 90% identity" or similar language refers to "at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or even 100% identity" to a specified reference sequence.

Signal Peptide: A short amino acid sequence (e.g., approximately 10-35 amino acids in length) that directs newly synthesized secretory or membrane proteins to and through membranes (for example, the endoplasmic reticulum membrane). Signal peptides are typically located at the N-terminus of a polypeptide and are removed by signal peptidases. Signal peptide sequences typically contain three common structural features: an N-terminal polar basic region (n-region), a hydrophobic core, and a hydrophilic c-region).

Single chain coronavirus S ectodomain: A recombinant coronavirus S ectodomain including the coronavirus S.sub.1 and S.sub.2 proteins in a single contiguous polypeptide chain. Single chain coronavirus S ectodomain can trimerize to form a coronavirus S ectodomain trimer. A single coronavirus S ectodomain includes mutations to prevent protease cleavage at the S.sub.1/S.sub.2 cleavage site and the S.sub.2' cleavage site in the S ectodomain. Therefore, when produced in cells, the S polypeptide is not cleaved into separate S.sub.1 and S.sub.2 polypeptide chains.

Soluble protein: A protein capable of dissolving in aqueous liquid at room temperature and remaining dissolved. The solubility of a protein may change depending on the concentration of the protein in the water-based liquid, the buffering condition of the liquid, the concentration of other solutes in the liquid, for example salt and protein concentrations, and the heat of the liquid. In several embodiments, a soluble protein is one that dissolves to a concentration of at least 0.5 mg/ml in phosphate buffered saline (pH 7.4) at room temperature and remains dissolved for at least 48 hours.

Subject: Living multi-cellular vertebrate organisms, a category that includes human and non-human mammals, such as non-human primates, pigs, camels, bats, sheep, cows, dogs, cats, rodents, and the like. In an example, a subject is a human. In a particular example, the subject is a camel or a bat. The subject can be a domestic animal (such as a dog or a cat) or a farm animal (such as a cow or a pig). In an additional example, a subject is selected that is in need of inhibiting of a coronavirus infection, such as a SARS-CoV or MERS-CoV infection. For example, the subject is either uninfected and at risk of the coronavirus infection or is infected and in need of treatment.

T4 Fibritin trimerization domain: Also referred to as a "foldon" domain, the T4 Fibritin trimerization domain comprises an amino acid sequence that naturally forms a trimeric structure. In some examples, a T4 Fibritin trimerization domain can be linked to the C-terminus of a disclosed recombinant coronavirus S protein ectodomain. In one example, a T4 Fibritin trimerization domain comprises the amino acid sequence set forth as (GYIPEAPRDGQAYVRKDGEWVLLSTF (SEQ ID NO: 22). In some embodiments, a protease cleavage site (such as a thrombin cleavage site) can be included between the C-terminus of the recombinant coronavirus ectodomain and the T4 Fibritin trimerization domain to facilitate removal of the trimerization domain as needed, for example, following expression and purification of the recombinant coronavirus S ectodomain.

Transmembrane domain: An amino acid sequence that inserts into a lipid bilayer, such as the lipid bilayer of a cell or virus or virus-like particle. A transmembrane domain can be used to anchor an antigen to a membrane. In some examples a transmembrane domain is a coronavirus S transmembrane domain, such as a MERS-CoV or SARS-CoV S transmembrane domain.

Vaccine: A pharmaceutical composition that induces a prophylactic or therapeutic immune response in a subject. In some cases, the immune response is a protective immune response. Typically, a vaccine induces an antigen-specific immune response to an antigen of a pathogen, for example a viral pathogen, or to a cellular constituent correlated with a pathological condition. A vaccine may include a polynucleotide (such as a nucleic acid encoding a disclosed antigen), a peptide or polypeptide (such as a disclosed antigen), a virus, a cell or one or more cellular constituents. In a non-limiting example, a vaccine induces an immune response that reduces the severity of the symptoms associated with a coronavirus infection (such as a SARS-CoV or MERS-CoV infection) and/or decreases the viral load compared to a control. In another non-limiting example, a vaccine induces an immune response that reduces and/or prevents a coronavirus infection (such as a SARS-CoV or MERS-CoV infection) compared to a control.

Vector: An entity containing a DNA or RNA molecule bearing a promoter(s) that is operationally linked to the coding sequence of an antigen(s) of interest and can express the coding sequence. Non-limiting examples include a naked or packaged (lipid and/or protein) DNA, a naked or packaged RNA, a subcomponent of a virus or bacterium or other microorganism that may be replication-incompetent, or a virus or bacterium or other microorganism that may be replication-competent. A vector is sometimes referred to as a

construct. Recombinant DNA vectors are vectors having recombinant DNA. A vector can include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector can also include one or more selectable marker genes and other genetic elements known in the art. Viral vectors are recombinant nucleic acid vectors having at least some nucleic acid sequences derived from one or more viruses.

Virus-like particle (VLP): A non-replicating, viral shell, derived from any of several viruses. VLPs are generally composed of one or more viral proteins, such as, but not limited to, those proteins referred to as capsid, coat, shell, surface and/or envelope proteins, or particle-forming polypeptides derived from these proteins. VLPs can form spontaneously upon recombinant expression of the protein in an appropriate expression system. Methods for producing particular VLPs are known in the art. The presence of VLPs following recombinant expression of viral proteins can be detected using conventional techniques known in the art, such as by electron microscopy, biophysical characterization, and the like. Further, VLPs can be isolated by known techniques, e.g., density gradient centrifugation and identified by characteristic density banding. See, for example, Baker et al. (1991) Biophys. J. 60:1445-1456; and Hagensee et al. (1994) J. Virol. 68:4503-4505; Vincente, J Invertebr Pathol., 2011; Schneider-Ohrum and Ross, Curr. Top. Microbiol. Immunol., 354: 53073, 2012).

II. Immunogens

Disclosed herein are recombinant coronavirus (such as alphacoronavirus or betacoronavirus) S ectodomain trimers comprising protomers comprising one or more proline substitution(s). The proline substitutions inhibit a conformational change in the S protein from the prefusion conformation to the postfusion conformation, and therefore stabilize the S ectodomain trimer in the prefusion conformation. In some embodiments, the recombinant coronavirus (such as alphacoronavirus or betacoronavirus) S ectodomain trimer comprises protomers comprising one or more (such as two) proline substitutions at or near the boundary between a HR1 domain and a central helix domain of the protomers. In some embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the protomers of the S ectodomain in the prefusion conformation are located between a position 15 amino acids N-terminal of a C-terminal residue of the HR1 and a position 5 amino acids C-terminal of a N-terminal residue of the central helix. Exemplary embodiments are shown to produce a superior immune response in an animal model compared to corresponding coronavirus S ectodomain trimers that are not stabilized in the prefusion conformation.

In some embodiments, the recombinant S ectodomain trimer comprises recombinant S ectodomain protomers from an alphacoronavirus, such as NL63-CoV or 229E-CoV, that have been mutated to include the one or more proline substitutions for stabilization in the prefusion conformation. In some embodiments, the recombinant S ectodomain trimers comprise S ectodomain protomers from a betacoronavirus, such as OC43-CoV, SARS-CoV, MERS-CoV, HKU1-CoV, WIV1-CoV, mouse hepatitis virus (MHV), or HKU9-CoV, that have been mutated to include the one or more proline substitutions for stabilization in the prefusion conformation. Additional description is provided below.

A. MERS-CoV

In some embodiments, the immunogen comprises a recombinant MERS-CoV S ectodomain trimer comprising protomers comprising one or more (such as two, for example two consecutive) proline substitutions at or near the boundary between a HR1 domain and a central helix domain that stabilize the S ectodomain trimer in the prefusion conformation. In some such embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the S ectodomain in the prefusion conformation are located between a position 15 amino acids N-terminal of a C-terminal residue of the HR1 and a position 5 amino acids C-terminal of a N-terminal residue of the central helix.

In some embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the MERS-CoV S ectodomain trimer in the prefusion conformation are located between residues 1050 to 1069 (such as between residues 1053 to 1063, or between residues 1058 to 1063) of the S ectodomain protomers in the trimer. In some embodiments, the MERS-CoV S ectodomain trimer is stabilized in the prefusion conformation by one or two of: L1058P, D1059P, V1060P, and L1061P substitutions in the S ectodomain protomers in the trimer. In some embodiments, the MERS-CoV S ectodomain trimer is stabilized in the prefusion conformation by V1060P and L1061P substitutions ("2P") in the S ectodomain protomers in the trimer. The amino acid numbering for MERS-CoV S proteins is with reference to the MERS-CoV S sequence provided as SEQ ID NO: 1.

In some embodiments, the recombinant MERS-CoV S ectodomain trimer stabilized in the prefusion conformation comprises protomers of single-chain S ectodomains comprising mutations to the S1/S2 and/or S2' protease cleavage sites to prevent protease cleavage at these sites. Non-limiting examples of such mutations include 748-RSVR-751 (residues 748-751 of SEQ ID NO: 1) to 748-ASVG-751 (residues 748-751 of SEQ ID NO: 3) substitutions to inhibit cleavage at the S1/S2 cleavage site, and 884-RSAR-887 (residues 884-887 of SEQ ID NO: 1) to 884-GSAG-887 (residues 884-887 of SEQ ID NO: 3) substitutions to inhibit cleavage at the S2' site.

In some embodiments, the recombinant MERS-CoV S ectodomain trimer comprising protomers stabilized in the prefusion conformation by the one or more proline substitutions (such as V1060P and L1061P substitutions) comprises additional modifications for stabilization in the prefusion conformation. In some embodiments, the recombinant MERS-CoV S ectodomain trimer comprising

protomers stabilized in the prefusion conformation by the one or more proline substitutions (such as V1060P and L1061P substitutions) further comprises cavity filling substitutions to stabilize the S ectodomain the prefusion conformation, such as one of: N1072F and A1083I; N1072F and L1086F; N1072F and V1087I; N1072F and E1090I; T1076F and A1083I; T1076F and L1086F; T1076F and V1087I; T1076F and E1090I; T1076I and A1083I; T1076I and L1086F; T1076I and V1087I; T1076I and E1090I; A1018V; or A1018I.

In some embodiments, the recombinant MERS-CoV S ectodomain trimer stabilized in the prefusion conformation by the one or more proline substitutions (such as V1060P and L1061P substitutions) further comprises a repacking substitution to stabilize the S ectodomain the prefusion conformation, such as one of: E793M and K1102F; E793M, K1102F, and H1138F; D1068M and R1069W; A1083L; A1083L and V1087I; A1083L, V1087, and E1090L; A834L and Q1084M; Q1066M; 5454F; R921W; S612F and G1052F; or P476V, T477A, and R1057W.

In some embodiments, the recombinant MERS-CoV S ectodomain trimer stabilized in the prefusion conformation by the one or more proline substitutions (such as V1060P and L1061P substitutions) further comprises one of A1083S, E1090I, Q1097I, D1101F, or A653W to stabilize the S ectodomain the prefusion conformation.

In some embodiments, the recombinant MERS-CoV S ectodomain trimer stabilized in the prefusion conformation by the one or more proline substitutions (such as V1060P and L1061P substitutions) further comprises a non-native disulfide bond formed between cysteines introduced by one of: T63C and V631C; T63C and Q638C; Q733C and D940C; S676C and D910C; V1087C (which forms a disulfide bond with a cysteine present in the native sequence); A432C and L1058C; or A432C and D1059C to stabilize the S ectodomain the prefusion conformation.

In some embodiments, the recombinant MERS-CoV S ectodomain trimer stabilized in the prefusion conformation by the one or more proline substitutions (such as V1060P and L1061P substitutions) further comprises an additional proline substitution to stabilize the S ectodomain the prefusion conformation, such as one of: K801P; V802P; T803P; V804P; S919P; A920P; A968P; A969P; I970P; F972P; A973P; T1014P; N1042P; T1043P; F1044P; G1045P; A1046P;I1047P; or A1049P.

Any of the substitutions described above can be combined in the MERS-CoV S ectodomain trimer, as long as the trimer is stabilized in the prefusion conformation and can be used to generate a neutralizing immune response to MERS-CoV in a subject.

With reference to the MERS-CoV S protein sequence provided as SEQ ID NO: 1, the ectodomain of the MERS-CoV S protein includes about residues 18-1291. Residues 1-17 are the signal peptide, which is removed during cellular processing. The S1/S2 cleavage site is located at about position 751/752. The S2' cleavage site is located at about position 887/888. The HR1 is located at about residues 989-1057. The central helix is located at about residues 1062-1103. The HR2 is located at about 1246-1277. The C-terminal end of the S2 ectodomain is located at about residue 1291. In some embodiments, the protomers of the prefusion-stabilized MERS-CoV S ectodomain trimer can have a C-terminal residue (which can be linked to a trimerization domain, or a transmembrane domain, for example) of the C-terminal residue of the HR2 (e.g., position 1277), or the ectodomain (e.g., position 1291) or from one of positions 1277-1291. The position numbering of the S protein may vary between MERS-CoV stains, but the sequences can be aligned to determine relevant structural domains and cleavage sites. It will be appreciated that a few residues (such as up to 10) on the N and C-terminal ends of the ectodomain can be removed or modified in the disclosed immunogens without decreasing the utility of the S ectodomain trimer as an immunogen.

Exemplary MERS-CoV S protein sequences are provided below. Any of the MERS-CoV S protein mutations (such as V1060P and L1061P, and/or modifications to generate a single chain) can be incorporated in the MERS-CoV S protein sequences.

An exemplary sequence of MERS-CoV S protein including the ectodomain and TM and CT domains) England1 strain is provided as SEQ ID NO: 1:

TABLE-US-00001 MIHSVFLLMFLLTPTESYVDVGPDSVKSACIEVDIQQTFFDKTWPRPIDV
SKADGIIYPQGRTYSNITITYQGLFPYQGDHGDMYVYSAGHATGTTPQKL
FVANYSQDVKQFANGFVVRIGAAANSTGTVIISPSTSATIRKIYPAFMLG
SSVGNFSDGKMGRFFNHTLVLLPDGCGTLLRAFYCILEPRSGNHCPAGNS
YTSFATYHTPATDCSDGNYNRNASLNSFKEYFNLRNCTFMYTYNITEDEI
LEWFGITQTAQGVHLFSSRYVDLYGGNMFQFATLPVYDTIKYYSIIPHSI
RSIQSDRKAWAAFYVYKLQPLTFLLDFSVDGYIRRAIDCGFNDLSQLHCS
YESFDVESGVYSVSSFEAKPSGSVVEQAEGVECDFSPLLSGTPPQVYNFK
RLVFTNCNYNLTKLLSLFSVNDFTCSQISPAAIASNCYSSLILDYFSYPL
SMKSDLSVSSAGPISQFNYKQSFSNPTCLILATVPHNLTTITKPLKYSYI
NKCSRFLSDDRTEVPQLVNANQYSPCVSIVPSTVWEDGDYYRKQLSPLEG
GGWLVASGSTVAMTEQLQMGFGITVQYGTDTNSVCPKLEFANDTKIASQL
GNCVEYSLYGVSGRGVFQNCTAVGVRQQRFVYDAYQNLVGYYSDDGNYYC
LRACVSVPVSVIYDKETKTHATLFGSVACEHISSTMSQYSRSTRSMLKRR
DSTYGPLQTPVGCVLGLVNSSLFVEDCKLPLGQSLCALPDTPSTLTPRSV
RSVPGEMRLASIAFNHPIQVDQLNSSYFKLSIPTNFSFGVTQEYIQTTIQ

KVTVDCKQYVCNGFQKCEQLLREYGQFCSKINQALHGANLRQDDSVRNLF
ASVKSSQSSPIIPGFGGDFNLTLLEPVSISTGSRSARSAIEDLLFDKVTI
ADPGYMQGYDDCMQQGPASARDLICAQYVAGYKVLPPLMDVNMEAAYTSS
LLGSIAGVGWTAGLSSFAAIPFAQSIFYRLNGVGITQQVLSENQKLIANK
FNQALGAMQTGFTTTNEAFHKVQDAVNNNAQALSKLASELSNTFGAISAS
IGDIIQRLDVLEQDAQIDRLINGRLTTLNAFVAQQLVRSESAALSAQLAK
DKVNECVKAQSKRSGFCGQGTHIVSFVVNAPNGLYFMHVGYYPSNHIEVV
SAYGLCDAANPTNCIAPVNGYFIKTNNTRIVDEWSYTGSSFYAPEPITSL
NTKYVAPQVTYQNISTNLPPPLLGNSTGIDFQDELDEFFKNVSTSIPNFG
SLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKELGNYTYYNKWPWYIW
LGFIAGLVALALCVFFILCCTGCGTNCMGKLKCNRCCDRYEEYDLEPHKV HVH

An exemplary sequence of MERS-CoV S ectodomain England1 strain including V1060P and L1061P substitutions is provided as SEQ ID NO: 2:

TABLE-US-00002 MIHSVFLLMFLLTPTESYVDVGPDSVKSACIEVDIQQTFFDKTWPRPIDV
SKADGIIYPQGRTYSNITITYQGLFPYQGDHGDMYVYSAGHATGTTPQKL
FVANYSQDVKQFANGFVVRIGAAANSTGTVIISPSTSATIRKIYPAFMLG
SSVGNFSDGKMGRFFNHTLVLLPDGCGTLLRAFYCILEPRSGNHCPAGNS
YTSFATYHTPATDCSDGNYNRNASLNSFKEYFNLRNCTFMYTYNITEDEI
LEWFGITQTAQGVHLFSSRYVDLYGGNMFQFATLPVYDTIKYYSIIPHSI
RSIQSDRKAWAAFYVYKLQPLTFLLDFSVDGYIRRAIDCGFNDLSQLHCS
YESFDVESGVYSVSSFEAKPSGSVVEQAEGVECDFSPLLSGTPPQVYNFK
RLVFTNCNYNLTKLLSLFSVNDFTCSQISPAAIASNCYSSLILDYFSYPL
SMKSDLSVSSAGPISQFNYKQSFSNPTCLILATVPHNLTTITKPLKYSYI
NKCSRFLSDDRTEVPQLVNANQYSPCVSIVPSTVWEDGDYYRKQLSPLEG
GGWLVASGSTVAMTEQLQMGFGITVQYGTDTNSVCPKLEFANDTKIASQL
GNCVEYSLYGVSGRGVFQNCTAVGVRQQRFVYDAYQNLVGYYSDDGNYYC
LRACVSVPVSVIYDKETKTHATLFGSVACEHISSTMSQYSRSTRSMLKRR
DSTYGPLQTPVGCVLGLVNSSLFVEDCKLPLGQSLCALPDTPSTLTPRSV
RSVPGEMRLASIAFNHPIQVDQLNSSYFKLSIPTNFSFGVTQEYIQTTIQ
KVTVDCKQYVCNGFQKCEQLLREYGQFCSKINQALHGANLRQDDSVRNLF
ASVKSSQSSPIIPGFGGDFNLTLLEPVSISTGSRSARSAIEDLLFDKVTI
ADPGYMQGYDDCMQQGPASARDLICAQYVAGYKVLPPLMDVNMEAAYTSS
LLGSIAGVGWTAGLSSFAAIPFAQSIFYRLNGVGITQQVLSENQKLIANK
FNQALGAMQTGFTTTNEAFHKVQDAVNNNAQALSKLASELSNTFGAISAS
IGDIIQRLDPPEQDAQIDRLINGRLTTLNAFVAQQLVRSESAALSAQLAK
DKVNECVKAQSKRSGFCGQGTHIVSFVVNAPNGLYFMHVGYYPSNHIEVV
SAYGLCDAANPTNCIAPVNGYFIKTNNTRIVDEWSYTGSSFYAPEPITSL
NTKYVAPQVTYQNISTNLPPPLLGNSTGIDFQDELDEFFKNVSTSIPNFG SLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKELGNYTY

An exemplary sequence of MERS-CoV S ectodomain England1 strain including V1060P and L1061P substitutions and 748-RSVR-751 (residues 748-751 of SEQ ID NO: 1) to 748-ASVG-751 (residues 748-751 of SEQ ID NO: 3) substitutions to remove the S1/S2 cleavage site is provided as SEQ ID NO: 3:

TABLE-US-00003 MIHSVFLLMFLLTPTESYVDVGPDSVKSACIEVDIQQTFFDKTWPRPIDV
SKADGIIYPQGRTYSNITITYQGLFPYQGDHGDMYVYSAGHATGTTPQKL
FVANYSQDVKQFANGFVVRIGAAANSTGTVIISPSTSATIRKIYPAFMLG
SSVGNFSDGKMGRFFNHTLVLLPDGCGTLLRAFYCILEPRSGNHCPAGNS
YTSFATYHTPATDCSDGNYNRNASLNSFKEYFNLRNCTFMYTYNITEDEI
LEWFGITQTAQGVHLFSSRYVDLYGGNMFQFATLPVYDTIKYYSIIPHSI
RSIQSDRKAWAAFYVYKLQPLTFLLDFSVDGYIRRAIDCGFNDLSQLHCS
YESFDVESGVYSVSSFEAKPSGSVVEQAEGVECDFSPLLSGTPPQVYNFK
RLVFTNCNYNLTKLLSLFSVNDFTCSQISPAAIASNCYSSLILDYFSYPL
SMKSDLSVSSAGPISQFNYKQSFSNPTCLILATVPHNLTTITKPLKYSYI
NKCSRFLSDDRTEVPQLVNANQYSPCVSIVPSTVWEDGDYYRKQLSPLEG
GGWLVASGSTVAMTEQLQMGFGITVQYGTDTNSVCPKLEFANDTKIASQL
GNCVEYSLYGVSGRGVFQNCTAVGVRQQRFVYDAYQNLVGYYSDDGNYYC
LRACVSVPVSVIYDKETKTHATLFGSVACEHISSTMSQYSRSTRSMLKRR
DSTYGPLQTPVGCVLGLVNSSLFVEDCKLPLGQSLCALPDTPSTLTPASV
GSVPGEMRLASIAFNHPIQVDQLNSSYFKLSIPTNFSFGVTQEYIQTTIQ
KVTVDCKQYVCNGFQKCEQLLREYGQFCSKINQALHGANLRQDDSVRNLF
ASVKSSQSSPIIPGFGGDFNLTLLEPVSISTGSRSARSAIEDLLFDKVTI
ADPGYMQGYDDCMQQGPASARDLICAQYVAGYKVLPPLMDVNMEAAYTSS
LLGSIAGVGWTAGLSSFAAIPFAQSIFYRLNGVGITQQVLSENQKLIANK
FNQALGAMQTGFTTTNEAFHKVQDAVNNNAQALSKLASELSNTFGAISAS
IGDIIQRLDPPEQDAQIDRLINGRLTTLNAFVAQQLVRSESAALSAQLAK
DKVNECVKAQSKRSGFCGQGTHIVSFVVNAPNGLYFMHVGYYPSNHIEVV

SAYGLCDAANPTNCIAPVNGYFIKTNNTRIVDEWSYTGSSFYAPEPITSL
NTKYVAPQVTYQNISTNLPPPLLGNSTGIDFQDELDEFFKNVSTSIPNFG SLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKELGNYTY

An exemplary sequence of MERS-CoV S ectodomain England1 strain including V1060P and L1061P substitutions and 748-RSVR-751 (residues 748-751 of SEQ ID NO: 1) to 748-ASVG-751 (residues 748-751 of SEQ ID NO: 3) and 884-RSAR-887 (residues 884-887 of SEQ ID NO: 1) to 884-GSAG-887 (residues 884-887 of SEQ ID NO: 3) substitutions to remove the S1/S2 cleavage site and the S2' cleavage site is provided as SEQ ID NO: 4:

TABLE-US-00004 MIHSVFLLMFLLTPTESYVDVGPDSVKSACIEVDIQQTFFDKTWPRPIDV
SKADGIIYPQGRTYSNITITYQGLFPYQGDHGDMYVYSAGHATGTTPQKL
FVANYSQDVKQFANGFVVRIGAAANSTGTVIISPSTSATIRKIYPAFMLG
SSVGNFSDGKMGRFFNHTLVLLPDGCGTLLRAFYCILEPRSGNHCPAGNS
YTSFATYHTPATDCSDGNYNRNASLNSFKEYFNLRNCTFMYTYNITEDEI
LEWFGITQTAQGVHLFSSRYVDLYGGNMFQFATLPVYDTIKYYSIIPHSI
RSIQSDRKAWAAFYVYKLQPLTFLLDFSVDGYIRRAIDCGFNDLSQLHCS
YESFDVESGVYSVSSFEAKPSGSVVEQAEGVECDFSPLLSGTPPQVYNFK
RLVFTNCNYNLTKLLSLFSVNDFTCSQISPAAIASNCYSSLILDYFSYPL
SMKSDLSVSSAGPISQFNYKQSFSNPTCLILATVPHNLTTITKPLKYSYI
NKCSRFLSDDRTEVPQLVNANQYSPCVSIVPSTVWEDGDYYRKQLSPLEG
GGWLVASGSTVAMTEQLQMGFGITVQYGTDTNSVCPKLEFANDTKIASQL
GNCVEYSLYGVSGRGVFQNCTAVGVRQQRFVYDAYQNLVGYYSDDGNYYC
LRACVSVPVSVIYDKETKTHATLFGSVACEHISSTMSQYSRSTRSMLKRR
DSTYGPLQTPVGCVLGLVNSSLFVEDCKLPLGQSLCALPDTPSTLTPASV
GSVPGEMRLASIAFNHPIQVDQLNSSYFKLSIPTNFSFGVTQEYIQTTIQ
KVTVDCKQYVCNGFQKCEQLLREYGQFCSKINQALHGANLRQDDSVRNLF
ASVKSSQSSPIIPGFGGDFNLTLLEPVSISTGSGSAGSAIEDLLFDKVTI
ADPGYMQGYDDCMQQGPASARDLICAQYVAGYKVLPPLMDVNMEAAYTSS
LLGSIAGVGWTAGLSSFAAIPFAQSIFYRLNGVGITQQVLSENQKLIANK
FNQALGAMQTGFTTTNEAFHKVQDAVNNNAQALSKLASELSNTFGAISAS
IGDIIQRLDPPEQDAQIDRLINGRLTTLNAFVAQQLVRSESAALSAQLAK
DKVNECVKAQSKRSGFCGQGTHIVSFVVNAPNGLYFMHVGYYPSNHIEVV
SAYGLCDAANPTNCIAPVNGYFIKTNNTRIVDEWSYTGSSFYAPEPITSL
NTKYVAPQVTYQNISTNLPPPLLGNSTGIDFQDELDEFFKNVSTSIPNFG
SLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKELGNYTYGGYIPEAPR DGQAYVRKDGEWVLLSTF

A C-terminal trimerization domain can be added to the protomers of the MERS-CoV S ectodomains trimer to promote trimerization of the ectodomain.

An exemplary sequence of MERS-CoV S ectodomain England1 strain including V1060P and L1061P substitutions and 748-RSVR-751 (residues 748-751 of SEQ ID NO: 1) to 748-ASVG-751 (residues 748-751 of SEQ ID NO: 3) substitutions to remove the S1/S2 cleavage site, and a T4 fibritin trimerization domain is provided as SEQ ID NO: 28:

TABLE-US-00005 MIHSVFLLMFLLTPTESYVDVGPDSVKSACIEVDIQQTFFDKTWPRPIDV
SKADGIIYPQGRTYSNITITYQGLFPYQGDHGDMYVYSAGHATGTTPQKL
FVANYSQDVKQFANGFVVRIGAAANSTGTVIISPSTSATIRKIYPAFMLG
SSVGNFSDGKMGRFFNHTLVLLPDGCGTLLRAFYCILEPRSGNHCPAGNS
YTSFATYHTPATDCSDGNYNRNASLNSFKEYFNLRNCTFMYTYNITEDEI
LEWFGITQTAQGVHLFSSRYVDLYGGNMFQFATLPVYDTIKYYSIIPHSI
RSIQSDRKAWAAFYVYKLQPLTFLLDFSVDGYIRRAIDCGFNDLSQLHCS
YESFDVESGVYSVSSFEAKPSGSVVEQAEGVECDFSPLLSGTPPQVYNFK
RLVFTNCNYNLTKLLSLFSVNDFTCSQISPAAIASNCYSSLILDYFSYPL
SMKSDLSVSSAGPISQFNYKQSFSNPTCLILATVPHNLTTITKPLKYSYI
NKCSRFLSDDRTEVPQLVNANQYSPCVSIVPSTVWEDGDYYRKQLSPLEG
GGWLVASGSTVAMTEQLQMGFGITVQYGTDTNSVCPKLEFANDTKIASQL
GNCVEYSLYGVSGRGVFQNCTAVGVRQQRFVYDAYQNLVGYYSDDGNYYC
LRACVSVPVSVIYDKETKTHATLFGSVACEHISSTMSQYSRSTRSMLKRR
DSTYGPLQTPVGCVLGLVNSSLFVEDCKLPLGQSLCALPDTPSTLTPASV
GSVPGEMRLASIAFNHPIQVDQLNSSYFKLSIPTNFSFGVTQEYIQTTIQ
KVTVDCKQYVCNGFQKCEQLLREYGQFCSKINQALHGANLRQDDSVRNLF
ASVKSSQSSPIIPGFGGDFNLTLLEPVSISTGSRSARSAIEDLLFDKVTI
ADPGYMQGYDDCMQQGPASARDLICAQYVAGYKVLPPLMDVNMEAAYTSS
LLGSIAGVGWTAGLSSFAAIPFAQSIFYRLNGVGITQQVLSENQKLIANK
FNQALGAMQTGFTTTNEAFHKVQDAVNNNAQALSKLASELSNTFGAISAS
IGDIIQRLDPPEQDAQIDRLINGRLTTLNAFVAQQLVRSESAALSAQLAK
DKVNECVKAQSKRSGFCGQGTHIVSFVVNAPNGLYFMHVGYYPSNHIEVV
SAYGLCDAANPTNCIAPVNGYFIKTNNTRIVDEWSYTGSSFYAPEPITSL
NTKYVAPQVTYQNISTNLPPPLLGNSTGIDFQDELDEFFKNVSTSIPNFG
SLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKELGNYTYGGYIPEAPR DGQAYVRKDGEWVLLSTF

An exemplary sequence of MERS-CoV S ectodomain England1 strain including V1060P and L1061P substitutions and 748-RSVR-751 to 748-ASVG-751 and 884-RSAR-887 (residues 884-887 of SEQ ID NO: 1) to 884-GSAG-887 (residues 884-887 of SEQ ID NO: 3) substitutions to remove the S1/S2 cleavage site and the S2' cleavage site, and a T4 fibritin trimerization domain is provided as SEQ ID NO: 29:

TABLE-US-00006 MIHSVFLLMFLLTPTESYVDVGPDSVKSACIEVDIQQTFFDKTWPRPIDV
SKADGIIYPQGRTYSNITITYQGLFPYQGDHGDMYVYSAGHATGTTPQKL
FVANYSQDVKQFANGFVVRIGAAANSTGTVIISPSTSATIRKIYPAFMLG
SSVGNFSDGKMGRFFNHTLVLLPDGCGTLLRAFYCILEPRSGNHCPAGNS
YTSFATYHTPATDCSDGNYNRNASLNSFKEYFNLRNCTFMYTYNITEDEI
LEWFGITQTAQGVHLFSSRYVDLYGGNMFQFATLPVYDTIKYYSIIPHSI
RSIQSDRKAWAAFYVYKLQPLTFLLDFSVDGYIRRAIDCGFNDLSQLHCS
YESFDVESGVYSVSSFEAKPSGSVVEQAEGVECDFSPLLSGTPPQVYNFK
RLVFTNCNYNLTKLLSLFSVNDFTCSQISPAAIASNCYSSLILDYFSYPL
SMKSDLSVSSAGPISQFNYKQSFSNPTCLILATVPHNLTTITKPLKYSYI
NKCSRFLSDDRTEVPQLVNANQYSPCVSIVPSTVWEDGDYYRKQLSPLEG
GGWLVASGSTVAMTEQLQMGFGITVQYGTDTNSVCPKLEFANDTKIASQL
GNCVEYSLYGVSGRGVFQNCTAVGVRQQRFVYDAYQNLVGYYSDDGNYYC
LRACVSVPVSVIYDKETKTHATLFGSVACEHISSTMSQYSRSTRSMLKRR
DSTYGPLQTPVGCVLGLVNSSLFVEDCKLPLGQSLCALPDTPSTLTPASV
GSVPGEMRLASIAFNHPIQVDQLNSSYFKLSIPTNFSFGVTQEYIQTTIQ
KVTVDCKQYVCNGFQKCEQLLREYGQFCSKINQALHGANLRQDDSVRNLF
ASVKSSQSSPIIPGFGGDFNLTLLEPVSISTGSGSAGSAIEDLLFDKVTI
ADPGYMQGYDDCMQQGPASARDLICAQYVAGYKVLPPLMDVNMEAAYTSS
LLGSIAGVGWTAGLSSFAAIPFAQSIFYRLNGVGITQQVLSENQKLIANK
FNQALGAMQTGFTTTNEAFHKVQDAVNNNAQALSKLASELSNTFGAISAS
IGDIIQRLDPPEQDAQIDRLINGRLTTLNAFVAQQLVRSESAALSAQLAK
DKVNECVKAQSKRSGFCGQGTHIVSFVVNAPNGLYFMHVGYYPSNHIEVV
SAYGLCDAANPTNCIAPVNGYFIKTNNTRIVDEWSYTGSSFYAPEPITSL
NTKYVAPQVTYQNISTNLPPPLLGNSTGIDFQDELDEFFKNVSTSIPNFG
SLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKELGNYTYGGYIPEAPR DGQAYVRKDGEWVLLSTF

In some embodiments, the recombinant MERS-CoV S ectodomain trimer comprises protomers comprising the ectodomain sequence of any one of SEQ ID NOs: 2-4 and 29. In some embodiments, the recombinant MERS-CoV S ectodomain trimer comprises protomers comprising residues 18-1291 of any one of SEQ ID NOs: 2-4 or residues 18-1318 of SEQ ID NO: 29. In some embodiments, the recombinant MERS-CoV S ectodomain trimer comprises protomers comprising an ectodomain sequence at least 90% identical to the ectodomain sequence of any one of SEQ ID NOs: 2-4, wherein the MERS-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers. In some embodiments, the recombinant MERS-CoV S ectodomain trimer comprises protomers comprising an amino acid sequence at least 90% identical to residues 18-1291 of any one of SEQ ID NOs: 2-4 or residues 18-1318 of SEQ ID NO: 29, wherein the MERS-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers.

B. SARS-CoV

In some embodiments, the immunogen comprises a recombinant SARS-CoV S ectodomain trimer comprising protomers comprising one or more (such as two, for example two consecutive) proline substitutions at or near the boundary between a HR1 domain and a central helix domain that stabilize the S ectodomain trimer in the prefusion conformation. In some such embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the S ectodomain in the prefusion conformation are located between a position 15 amino acids N-terminal of a C-terminal residue of the HR1 and a position 5 amino acids C-terminal of a N-terminal residue of the central helix.

In some embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the SARS-CoV S ectodomain trimer in the prefusion conformation are located between residues 951 to 971 (such as between residues 961 to 971 or between residues 966 to 971) of the S ectodomain protomers in the trimer. In some embodiments, the SARS-CoV S ectodomain trimer is stabilized in the prefusion conformation by K968P and V969P substitutions ("2P") in the S ectodomain protomers in the trimer. The amino acid numbering for SARS-CoV S proteins is with reference to the SARS-CoV S sequence provided as SEQ ID NO: 6.

In some embodiments, the recombinant SARS-CoV S ectodomain trimer stabilized in the prefusion conformation comprises single-chain S ectodomain protomers comprising mutations to the S1/S2 and/or S2' protease cleavage sites to prevent protease cleavage at these sites.

In some embodiments, the protomers of the recombinant SARS-CoV S ectodomain trimer stabilized in the prefusion conformation by the one or more proline substitutions (such as K968P and V969P substitutions) comprises additional modifications for stabilization in the prefusion conformation.

With reference to the SARS-CoV S protein sequence provided as SEQ ID NO: 6, the ectodomain of the SARS-CoV S protein includes about residues 14-1190. Residues 1-13 are the signal peptide, which is removed during cellular processing. The S1/S2 cleavage site is located at position 667/668 or 678/679. The S2' cleavage site is located at about position 797/798. The HR1 is located at about residues 897-965. The central helix is located at about residues 970-1011. The HR2 is located at about 1145-1176. The C-terminal end of the S2 ectodomain is located at about residue 1190. In some embodiments, the protomers of the prefusion-stabilized SARS-CoV S ectodomain trimer can have a C-terminal residue (which can be linked to a trimerization domain, or a transmembrane domain, for example) of the C-terminal residue of the HR2 (e.g., position 1176), or the ectodomain (e.g., position 1190), or from one of positions 1176-1190. The position numbering of the S protein may vary between SARS-CoV stains, but the sequences can be aligned to determine relevant structural domains and cleavage sites. It will be appreciated that a few residues (such as up to 10) on the N and C-terminal ends of the ectodomain can be removed or modified in the disclosed immunogens without decreasing the utility of the S ectodomain trimer as an immunogen.

Exemplary SARS-CoV S protein sequences are provided below. The prefusion stabilizing substitutions disclosed herein (and other modifications, such as substitutions to generate a single chain) can be incorporated into SARS-CoV S protein sequences.

An exemplary sequence of SARS-CoV S protein (including the ectodomain and TM and CT domains) is provided as SEQ ID NO: 6 (GenBank GI: 30795145, incorporated by reference herein):

TABLE-US-00007 MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSD
TLYLTQDLFLPFYSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRG
WVFGSTMNNKSQSVIIINNSTNVVIRACNFELCDNPFFAVSKPMGTQTHT
MIFDNAFNCTFEYISDAFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGY
QPIDVVRDLPSGFNTLKPIFKLPLGINITNFRAILTAFSPAQDIWGTSAA
AYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAELKCSVKSFEIDKGIY
QTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVA
DYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPG
QTGVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRP
FERDISNVPFSPDGKPCTPPALNCYWPLNDYGFYTTTGIGYQPYRVVVLS
FELLNAPATVCGPKLSTDLIKNQCVNFNFNGLTGTGVLTPSSKRFQPFQQ
FGRDVSDFTDSVRDPKTSEILDISPCAFGGVSVITPGTNASSEVAVLYQD
VNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHVDTSYECDI
PIGAGICASYHTVSLLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTNF
SISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALS
GIAAEQDRNTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFI
EDLLFNKVTLADAGFMKQYGECLGDINARDLICAQKFNGLTVLPPLLTDD
MIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYE
NQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSS
NFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEI
RASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTYV
PSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITTD
NTFVSGNCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGD
ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYVWL
GFIAGLIAIVMVTILLCCMTSCCSCLKGACSCGSCCKFDEDDSEPVLKGV KLHYT

An exemplary sequence of SARS-CoV S ectodomain (TOR2 strain) including a double proline substitution for stabilization in the prefusion conformation is provided as SEQ ID NO: 7:

TABLE-US-00008 MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSD
TLYLTQDLFLPFYSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRG
WVFGSTMNNKSQSVIIINNSTNVVIRACNFELCDNPFFAVSKPMGTQTHT
MIFDNAFNCTFEYISDAFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGY
QPIDVVRDLPSGFNTLKPIFKLPLGINITNFRAILTAFSPAQDIWGTSAA
AYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAELKCSVKSFEIDKGIY
QTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVA
DYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPG
QTGVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRP
FERDISNVPFSPDGKPCTPPALNCYWPLNDYGFYTTTGIGYQPYRVVVLS
FELLNAPATVCGPKLSTDLIKNQCVNFNFNGLTGTGVLTPSSKRFQPFQQ
FGRDVSDFTDSVRDPKTSEILDISPCAFGGVSVITPGTNASSEVAVLYQD
VNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHVDTSYECDI
PIGAGICASYHTVSLLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTNF
SISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALS
GIAAEQDRNTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFI
EDLLFNKVTLADAGFMKQYGECLGDINARDLICAQKFNGLTVLPPLLTDD
MIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYE
NQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSS

NFGAISSVLNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEI
RASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTYV
PSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITTD
NTFVSGNCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGD ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQ

A C-terminal trimerization domain can be added to the protomers of the SARS-CoV S ectodomains trimer to promote trimerization of the ectodomain.

An exemplary sequence of SARS-CoV S ectodomain (TOR2 strain) including a double proline substitution for stabilization in the prefusion conformation, and a T4 fibritin trimerization domain is provided as SEQ ID NO: 30:

TABLE-US-00009 MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSD
TLYLTQDLFLPFYSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRG
WVFGSTMNNKSQSVIIINNSTNVVIRACNFELCDNPFFAVSKPMGTQTHT
MIFDNAFNCTFEYISDAFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGY
QPIDVVRDLPSGFNTLKPIFKLPLGINITNFRAILTAFSPAQDIWGTSAA
AYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAELKCSVKSFEIDKGIY
QTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVA
DYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPG
QTGVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRP
FERDISNVPFSPDGKPCTPPALNCYWPLNDYGFYTTTGIGYQPYRVVVLS
FELLNAPATVCGPKLSTDLIKNQCVNFNFNGLTGTGVLTPSSKRFQPFQQ
FGRDVSDFTDSVRDPKTSEILDISPCAFGGVSVITPGTNASSEVAVLYQD
VNCTDVSTAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHVDTSYECDI
PIGAGICASYHTVSLLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTNF
SISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALS
GIAAEQDRNTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFI
EDLLFNKVTLADAGFMKQYGECLGDINARDLICAQKFNGLTVLPPLLTDD
MIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYE
NQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSS
NFGAISSVLNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEI
RASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTYV
PSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITTD
NTFVSGNCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGD
ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQGGYIPEAPRD GQAYVRKDGEWVLLSTF

In some embodiments, the recombinant SARS-CoV S ectodomain trimer comprises protomers comprising the ectodomain sequence of SEQ ID NO: 7. In some embodiments, the recombinant SARS-CoV S ectodomain trimer comprises protomers comprising residues 14-1190 of SEQ ID NO: 7 or residues 14-1217 of SEQ ID NO: 30. In some embodiments, the recombinant SARS-CoV S ectodomain trimer comprises protomers comprising an ectodomain sequence at least 90% identical to the ectodomain sequence of SEQ ID NO: 7, wherein the SARS-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers. In some embodiments, the recombinant SARS-CoV S ectodomain trimer comprises protomers comprising an amino acid sequence at least 90% identical to residues 14-1190 of SEQ ID NO: 7, wherein the SARS-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers.

C. HKU1-CoV

In some embodiments, the immunogen comprises a recombinant HKU1-CoV S ectodomain trimer comprising protomers comprising one or more (such as two, for example two consecutive) proline substitutions at or near the boundary between a HR1 domain and a central helix domain that stabilize the S ectodomain trimer in the prefusion conformation. In some such embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the S ectodomain in the prefusion conformation are located between a position 15 amino acids N-terminal of a C-terminal residue of the HR1 and a position 5 amino acids C-terminal of a N-terminal residue of the central helix.

In some embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the HKU1-CoV S ectodomain trimer in the prefusion conformation are located between residues 1050 to 1070 (such as between residues 1060 to 1070 or between residues 1065 to 1070) of the S ectodomain protomers in the trimer. In some embodiments, the HKU1-CoV S ectodomain trimer is stabilized in the prefusion conformation by N1067P and L1068P substitutions ("2P") in the S ectodomain protomers in the trimer. The amino acid numbering for HKU1-CoV S proteins is with reference to the HKU1-CoV S sequence provided as SEQ ID NO: 7.

In some embodiments, the recombinant HKU1-CoV S ectodomain trimer stabilized in the prefusion conformation comprises single-chain S ectodomain protomers comprising mutations to the S1/S2 and/or S2' protease cleavage sites to prevent protease cleavage at these sites.

In some embodiments, the protomers of the recombinant HKU1-CoV S ectodomain trimer stabilized in the prefusion conformation by the one or more proline substitutions (such as N1067P and L1068P substitutions) comprise additional modifications for stabilization in the prefusion conformation.

With reference to the HKU1-CoV S protein sequence provided as SEQ ID NO: 8, the ectodomain of the HKU1-CoV S protein includes about residues 14-1290. Residues 1-13 are the signal peptide, which is removed during cellular processing. The S1/S2 cleavage site is located at about position 756/757. The S2' cleavage site is located at about position 900/901. The HR1 is located at about residues 996-1064. The central helix is located at about residues 1069-1110. The HR2 is located at about 1245-1276. The C-terminal end of the S2 ectodomain is located at about residue 1290. In some embodiments, the protomers of the prefusion-stabilized HKU1-CoV S ectodomain trimer can have a C-terminal residue (which can be linked to a trimerization domain, or a transmembrane domain, for example) of the C-terminal residue of the HR2 (e.g., position 1276), or the ectodomain (e.g., position 1290), or from one of positions 1276-1290. The position numbering of the S protein may vary between HKU1-CoV stains, but the sequences can be aligned to determine relevant structural domains and cleavage sites. It will be appreciated that a few residues (such as up to 10) on the N and C-terminal ends of the ectodomain can be removed or modified in the disclosed immunogens without decreasing the utility of the S ectodomain trimer as an immunogen.

Exemplary HKU1-CoV S protein sequences are provided below. The prefusion stabilizing substitutions disclosed herein (and other modifications, such as substitutions to generate a single chain) can be incorporated into HKU1-CoV S protein sequences.

An exemplary sequence of HKU1-CoV S protein (including the ectodomain and TM and CT domains) is provided as SEQ ID NO: 8 (GenBank GI: 123867264, incorporated by reference herein):

TABLE-US-00010 MFLIIFILPTTLAVIGDFNCTNSFINDYNKTIPRISEDVVDVSLGLGTYY
VLNRVYLNTTLLFTGYFPKSGANFRDLALKGSIYLSTLWYKPPFLSDFNN
GIFSKVKNTKLYVNNTLYSEFSTIVIGSVFVNTSYTIVVQPHNGILEITA
CQYTMCEYPHTVCKSKGSIRNESWHIDSSEPLCLFKKNFTYNVSADWLYF
HFYQERGVFYAYYADVGMPTTFLFSLYLGTILSHYYVMPLTCNAISSNTD
NETLEYWVTPLSRRQYLLNFDEHGVITNAVDCSSSFLSEIQCKTQSFAPN
TGVYDLSGFTVKPVATVYRRIPNLPDCDIDNWLNNVSVPSPLNWERRIFS
NCNFNLSTLLRLVHVDSFSCNNLDKSKIFGSCFNSITVDKFAIPNRRRDD
LQLGSSGFLQSSNYKIDISSSSCQLYYSLPLVNVTINNFNPSSWNRRYGF
GSFNLSSYDVVYSDHCFSVNSDFCPCADPSVVNSCAKSKPPSAICPAGTK
YRHCDLDTTLYVKNWCRCSCLPDPISTYSPNTCPQKKVVVGIGEHCPGLG
INEEKCGTQLNHSSCFCSPDAFLGWSFDSCISNNRCNIFSNFIFNGINSG
TTCSNDLLYSNTEISTGVCVNYDLYGITGQGIFKEVSAAYYNNWQNLLYD
SNGNIIGFKDFLTNKTYTILPCYSGRVSAAFYQNSSSPALLYRNLKCSYV
LNNISFISQPFYFDSYLGCVLNAVNLTSYSVSSCDLRMGSGFCIDYALPS
SRRKRRGISSPYRFVTFEPFNVSFVNDSVETVGGLFEIQIPTNFTIAGHE
EFIQTSSPKVTIDCSAFVCSNYAACHDLLSEYGTFCDNINSILNEVNDLL
DITQLQVANALMQGVTLSSNLNTNLHSDVDNIDFKSLLGCLGSQCGSSSR
SLLEDLLFNKVKLSDVGFVEAYNNCTGGSEIRDLLCVQSFNGIKVLPPIL
SETQISGYTTAATVAAMFPPWSAAAGVPFSLNVQYRINGLGVTMDVLNKN
QKLIANAFNKALLSIQNGFTATNSALAKIQSVVNANAQALNSLLQQLFNK
FGAISSSLQEILSRLDNLEAQVQIDRLINGRLTALNAYVSQQLSDITLIK
AGASRAIEKVNECVKSQSPRINFCGNGNHILSLVQNAPYGLLFIHFSYKP
TSFKTVLVSPGLCLSGDRGIAPKQGYFIKQNDSWMFTGSSYYYPEPISDK
NVVFMNSCSVNFTKAPFIYLNNSIPNLSDFEAELSLWFKNHTSIAPNLTF
NSHINATFLDLYYEMNVIQESIKSLNSSFINLKEIGTYEMYVKWPWYIWL
LIVILFIIFLMILFFICCCTGCGSACFSKCHNCCDEYGGHNDFVIKASHD D

An exemplary sequence of HKU1-CoV S ectodomain including a double proline substitution for stabilization in the prefusion conformation is provided as (SEQ ID NO: 9, which also includes mutations to eliminate the S1/S2 cleavage site:

TABLE-US-00011 MFLIIFILPTTLAVIGDFNCTNSFINDYNKTIPRISEDVVDVSLGLGTYY
VLNRVYLNTTLLFTGYFPKSGANFRDLALKGSIYLSTLWYKPPFLSDFNN
GIFSKVKNTKLYVNNTLYSEFSTIVIGSVFVNTSYTIVVQPHNGILEITA
CQYTMCEYPHTVCKSKGSIRNESWHIDSSEPLCLFKKNFTYNVSADWLYF
HFYQERGVFYAYYADVGMPTTFLFSLYLGTILSHYYVMPLTCNAISSNTD
NETLEYWVTPLSRRQYLLNFDEHGVITNAVDCSSSFLSEIQCKTQSFAPN
TGVYDLSGFTVKPVATVYRRIPNLPDCDIDNWLNNVSVPSPLNWERRIFS
NCNFNLSTLLRLVHVDSFSCNNLDKSKIFGSCFNSITVDKFAIPNRRRDD
LQLGSSGFLQSSNYKIDISSSSCQLYYSLPLVNVTINNFNPSSWNRRYGF
GSFNLSSYDVVYSDHCFSVNSDFCPCADPSVVNSCAKSKPPSAICPAGTK
YRHCDLDTTLYVKNWCRCSCLPDPISTYSPNTCPQKKVVVGIGEHCPGLG
INEEKCGTQLNHSSCFCSPDAFLGWSFDSCISNNRCNIFSNFIFNGINSG

TTCSNDLLYSNTEISTGVCVNYDLYGITGQGIFKEVSAAYYNNWQNLLYD
SNGNIIGFKDFLTNKTYTILPCYSGRVSAAFYQNSSSPALLYRNLKCSYV
LNNISFISQPFYFDSYLGCVLNAVNLTSYSVSSCDLRMGSGFCIDYALPS
SGGSGSGISSPYRFVTFEPFNVSFVNDSVETVGGLFEIQIPTNFTIAGHE
EFIQTSSPKVTIDCSAFVCSNYAACHDLLSEYGTFCDNINSILNEVNDLL
DITQLQVANALMQGVTLSSNLNTNLHSDVDNIDFKSLLGCLGSQCGSSSR
SLLEDLLFNKVKLSDVGFVEAYNNCTGGSEIRDLLCVQSFNGIKVLPPIL
SETQISGYTTAATVAAMFPPWSAAAGVPFSLNVQYRINGLGVTMDVLNKN
QKLIANAFNKALLSIQNGFTATNSALAKIQSVVNANAQALNSLLQQLFNK
FGAISSSLQEILSRLDPPEAQVQIDRLINGRLTALNAYVSQQLSDITLIK
AGASRAIEKVNECVKSQSPRINFCGNGNHILSLVQNAPYGLLFIHFSYKP
TSFKTVLVSPGLCLSGDRGIAPKQGYFIKQNDSWMFTGSSYYYPEPISDK
NVVFMNSCSVNFTKAPFIYLNNSIPNLSDFEAELSLWFKNHTSIAPNLTF NSHINATFLDLYYEMNVIQESIKSLN

A C-terminal trimerization domain can be added to the protomers of the HKU1-CoV S ectodomains trimer to promote trimerization of the ectodomain.

An exemplary sequence of HKU1-CoV S ectodomain including a double proline substitution for stabilization in the prefusion conformation, mutations to eliminate the S1/S2 cleavage site, and a T4 fibritin trimerization domain is provided as SEQ ID NO: 31:

TABLE-US-00012 MFLIIFILPTTLAVIGDFNCTNSFINDYNKTIPRISEDVVDVSLGLGTYY
VLNRVYLNTTLLFTGYFPKSGANFRDLALKGSIYLSTLWYKPPFLSDFNN
GIFSKVKNTKLYVNNTLYSEFSTIVIGSVFVNTSYTIVVQPHNGILEITA
CQYTMCEYPHTVCKSKGSIRNESWHIDSSEPLCLFKKNFTYNVSADWLYF
HFYQERGVFYAYYADVGMPTTFLFSLYLGTILSHYYVMPLTCNAISSNTD
NETLEYWVTPLSRRQYLLNFDEHGVITNAVDCSSSFLSEIQCKTQSFAPN
TGVYDLSGFTVKPVATVYRRIPNLPDCDIDNWLNNVSVPSPLNWERRIFS
NCNFNLSTLLRLVHVDSFSCNNLDKSKIFGSCFNSITVDKFAIPNRRRDD
LQLGSSGFLQSSNYKIDISSSSCQLYYSLPLVNVTINNFNPSSWNRRYGF
GSFNLSSYDVVYSDHCFSVNSDFCPCADPSVVNSCAKSKPPSAICPAGTK
YRHCDLDTTLYVKNWCRCSCLPDPISTYSPNTCPQKKVVVGIGEHCPGLG
INEEKCGTQLNHSSCFCSPDAFLGWSFDSCISNNRCNIFSNFIFNGINSG
TTCSNDLLYSNTEISTGVCVNYDLYGITGQGIFKEVSAAYYNNWQNLLYD
SNGNIIGFKDFLTNKTYTILPCYSGRVSAAFYQNSSSPALLYRNLKCSYV
LNNISFISQPFYFDSYLGCVLNAVNLTSYSVSSCDLRMGSGFCIDYALPS
SGGSGSGISSPYRFVTFEPFNVSFVNDSVETVGGLFEIQIPTNFTIAGHE
EFIQTSSPKVTIDCSAFVCSNYAACHDLLSEYGTFCDNINSILNEVNDLL
DITQLQVANALMQGVTLSSNLNTNLHSDVDNIDFKSLLGCLGSQCGSSSR
SLLEDLLFNKVKLSDVGFVEAYNNCTGGSEIRDLLCVQSFNGIKVLPPIL
SETQISGYTTAATVAAMFPPWSAAAGVPFSLNVQYRINGLGVTMDVLNKN
QKLIANAFNKALLSIQNGFTATNSALAKIQSVVNANAQALNSLLQQLFNK
FGAISSSLQEILSRLDPPEAQVQIDRLINGRLTALNAYVSQQLSDITLIK
AGASRAIEKVNECVKSQSPRINFCGNGNHILSLVQNAPYGLLFIHFSYKP
TSFKTVLVSPGLCLSGDRGIAPKQGYFIKQNDSWMFTGSSYYYPEPISDK
NVVFMNSCSVNFTKAPFIYLNNSIPNLSDFEAELSLWFKNHTSIAPNLTF
NSHINATFLDLYYEMNVIQESIKSLNGGYIPEAPRDGQAYVRKDGEWVLL STF

In some embodiments, the recombinant HKU1-CoV S ectodomain trimer comprises protomers comprising the ectodomain sequence of SEQ ID NO: 9. In some embodiments, the recombinant HKU1-CoV S ectodomain trimer comprises protomers comprising residues 14-1276 of SEQ ID NO: 9 or residues 14-1303 of SEQ ID NO: 31. In some embodiments, the recombinant HKU1-CoV S ectodomain trimer comprises protomers comprising an ectodomain sequence at least 90% identical to the ectodomain sequence of SEQ ID NO: 9, wherein the HKU1-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers. In some embodiments, the recombinant HKU1-CoV S ectodomain trimer comprises protomers comprising an amino acid sequence at least 90% identical to residues 14-1276 of SEQ ID NO: 9 or residues 14-1303 of SEQ ID NO: 31, wherein the HKU1-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers.

D. HKU9-CoV

In some embodiments, the immunogen comprises a recombinant HKU9-CoV S ectodomain trimer comprising protomers comprising one or more (such as two, for example two consecutive) proline substitutions at or near the boundary between a HR1 domain and a central helix domain that stabilize the S ectodomain trimer in the prefusion conformation. In some such embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the S ectodomain in the prefusion conformation are located between a position 15 amino acids N-terminal of a C-terminal residue of the HR1 and a position 5 amino acids C-terminal of a N-terminal residue of the central helix.

In some embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the HKU9-CoV S ectodomain trimer in the prefusion conformation are located between residues 966 to 986 (such as between residues 976 to 986 or between residues 981 to 986) of the S ectodomain protomers in the trimer. In some embodiments, the HKU9-CoV S ectodomain trimer is stabilized in the prefusion conformation by G1018P and L1019P substitutions ("2P") in the S ectodomain protomers in the trimer. The amino acid numbering for HKU9-CoV S proteins is with reference to the HKU9-CoV S sequence provided as SEQ ID NO: 12.

In some embodiments, the recombinant HKU9-CoV S ectodomain trimer stabilized in the prefusion conformation comprises single-chain S ectodomain protomers comprising mutations to the S1/S2 and/or S2' protease cleavage sites to prevent protease cleavage at these sites.

In some embodiments, the protomers of the recombinant HKU9-CoV S ectodomain trimer stabilized in the prefusion conformation by the one or more proline substitutions (such as G1018P and L1019P substitutions) comprises additional modifications for stabilization in the prefusion conformation.

With reference to the HKU9-CoV S protein sequence provided as SEQ ID NO: 12, the ectodomain of the HKU9-CoV S protein includes about residues 15-1207. Residues 1-14 are the signal peptide, which is removed during cellular processing. The S1/S2 cleavage site is located at about position 676/677. The S2' cleavage site is located at about position 809/810. The HR1 is located at about residues 912-980. The central helix is located at about residues 986-1026. The HR2 is located at about 1162-1193. The C-terminal end of the S2 ectodomain is located at about residue 1207. In some embodiments, the protomers of the prefusion-stabilized HKU9-CoV S ectodomain trimer can have a C-terminal residue (which can be linked to a trimerization domain, or a transmembrane domain, for example) of the C-terminal residue of the HR2 (e.g., position 1193), or the ectodomain (e.g., position 1207), or from one of positions 1193-1207. The position numbering of the S protein may vary between HKU9-CoV stains, but the sequences can be aligned to determine relevant structural domains and cleavage sites. It will be appreciated that a few residues (such as up to 10) on the N and C-terminal ends of the ectodomain can be removed or modified in the disclosed immunogens without decreasing the utility of the S ectodomain trimer as an immunogen.

Exemplary HKU9-CoV S protein sequences are provided below. The prefusion stabilizing substitutions disclosed herein (and other modifications, such as substitutions to generate a single chain) can be incorporated into HKU9-CoV S protein sequences.

An exemplary sequence of HKU9-CoV S protein (including the ectodomain and TM and CT domains) is provided as SEQ ID NO: 12 (GenBank GI:148841195, incorporated by reference herein):

TABLE-US-00013 MLLILVLGVSLAAASRPECFNPRFTLTPLNHTLNYTSIKAKVSNVLLPDP
YIAYSGQTLRQNLFMADMSNTILYPVTPPANGANGGFIYNTSIIPVSAGL
FVNTWMYRQPASSRAYCQEPFGVAFGDTFENDRIAILIMAPDNLGSWSAV
APRNQTNIYLLVCSNATLCINPGFNRWGPAGSFIAPDALVDHSNSCFVNN
TFSVNISTSRISLAFLFKDGDLLIYHSGWLPTSNFEHGFSRGSHPMTYFM
SLPVGGNLPRAQFFQSIVRSNAIDKGDGMCTNFDVNLHVAHLINRDLLVS
YFNNGSVANAADCADSAAEELYCVTGSFDPPTGVYPLSRYRAQVAGFVRV
TQRGSYCTPPYSVLQDPPQPVVWRRYMLYDCVFDFTVVVDSLPTHQLQCY
GVSPRRLASMCYGSVTLDVMRINETHLNNLFNRVPDTFSLYNYALPDNFY
GCLHAFYLNSTAPYAVANRFPIKPGGRQSNSAFIDTVINAAHYSPFSYVY
GLAVITLKPAAGSKLVCPVANDTVVITDRCVQYNLYGYTGTGVLSKNTSL
VIPDGKVFTASSTGTIIGVSINSTTYSIMPCVTVPVSVGYHPNFERALLF
NGLSCSQRSRAVTEPVSVLWSASATAQDAFDTPSGCVVNVELRNTTIVNT
CAMPIGNSLCFINGSIATANADSLPRLQLVNYDPLYDNSTATPMTPVYWV
KVPTNFTLSATEEYIQTTAPKITIDCARYLCGDSSRCLNVLLHYGTFCND
INKALSRVSTILDSALLSLVKELSINTRDEVTTFSFDGDYNFTGLMGCLG
PNCGATTYRSAFSDLLYDKVRITDPGFMQSYQKCIDSQWGGSIRDLLCTQ
TYNGIAVLPPIVSPAMQALYTSLLVGAVASSGYTFGITSAGVIPFATQLQ
FRLNGIGVTTQVLVENQKLIASSFNNALVNIQKGFTETSIALSKMQDVIN
QHAAQLHTLVVQLGNSFGAISSSINEIFSRLEGLAANAEVDRLINGRMMV
LNTYVTQLLIQASEAKAQNALAAQKISECVKAQSLRNDFCGNGTHVLSIP
QLAPNGVLFIHYAYTPTEYAFVQTSAGLCHNGTGYAPRQGMFVLPNNTNM
WHFTTMQFYNPVNISASNTQVLTSCSVNYTSVNYTVLEPSVPGDYDFQKE
FDKFYKNLSTIFNNTFNPNDFNFSTVDVTAQIKSLHDVVNQLNQSFIDLK
KLNVYEKTIKWPWYVWLAMIAGIVGLVLAVIMLMCMTNCCSCFKGMCDCR RCCGSYDSYDDVYPAVRVNKKRTV

An exemplary sequence of HKU9-CoV S protein including a double proline substitution for stabilization in the prefusion conformation is provided as SEQ ID NO: 13:

TABLE-US-00014 MLLILVLGVSLAAASRPECFNPRFTLTPLNHTLNYTSIKAKVSNVLLPDP
YIAYSGQTLRQNLFMADMSNTILYPVTPPANGANGGFIYNTSIIPVSAGL

FVNTWMYRQPASSRAYCQEPFGVAFGDTFENDRIAILIMAPDNLGSWSAV
APRNQTNIYLLVCSNATLCINPGFNRWGPAGSFIAPDALVDHSNSCFVNN
TFSVNISTSRISLAFLFKDGDLLIYHSGWLPTSNFEHGFSRGSHPMTYFM
SLPVGGNLPRAQFFQSIVRSNAIDKGDGMCTNFDVNLHVAHLINRDLLVS
YFNNGSVANAADCADSAAEELYCVTGSFDPPTGVYPLSRYRAQVAGFVRV
TQRGSYCTPPYSVLQDPPQPVVWRRYMLYDCVFDFTVVVDSLPTHQLQCY
GVSPRRLASMCYGSVTLDVMRINETHLNNLFNRVPDTFSLYNYALPDNFY
GCLHAFYLNSTAPYAVANRFPIKPGGRQSNSAFIDTVINAAHYSPFSYVY
GLAVITLKPAAGSKLVCPVANDTVVITDRCVQYNLYGYTGTGVLSKNTSL
VIPDGKVFTASSTGTIIGVSINSTTYSIMPCVTVPVSVGYHPNFERALLF
NGLSCSQRSRAVTEPVSVLWSASATAQDAFDTPSGCVVNVELRNTTIVNT
CAMPIGNSLCFINGSIATANADSLPRLQLVNYDPLYDNSTATPMTPVYWV
KVPTNFTLSATEEYIQTTAPKITIDCARYLCGDSSRCLNVLLHYGTFCND
INKALSRVSTILDSALLSLVKELSINTRDEVTTFSFDGDYNFTGLMGCLG
PNCGATTYRSAFSDLLYDKVRITDPGFMQSYQKCIDSQWGGSIRDLLCTQ
TYNGIAVLPPIVSPAMQALYTSLLVGAVASSGYTFGITSAGVIPFATQLQ
FRLNGIGVTTQVLVENQKLIASSFNNALVNIQKGFTETSIALSKMQDVIN
QHAAQLHTLVVQLGNSFGAISSSINEIFSRLEPPAANAEVDRLINGRMMV
LNTYVTQLLIQASEAKQNALAAQKISECVKAQSLRNDFCGNGTHVLSIP
QLAPNGVLFIHYAYTPTEYAFVQTSAGLCHNGTGYAPRQGMFVLPNNTNM
WHFTTMQFYNPVNISASNTQVLTSCSVNYTSVNYTVLEPSVPGDYDFQKE
FDKFYKNLSTIFNNTFNPNDFNFSTVDVTAQIKSLHDVVNQLNQSFIDLK KLNVYEK

A C-terminal trimerization domain can be added to the protomers of the HKU9-CoV S ectodomains trimer to promote trimerization of the ectodomain.

An exemplary sequence of HKU9-CoV S protein including a double proline substitution for stabilization in the prefusion conformation, and a T4 fibritin trimerization domain is provided as SEQ ID NO: 32:

TABLE-US-00015 MLLILVLGVSLAAASRPECFNPRFTLTPLNHTLNYTSIKAKVSNVLLPDP
YIAYSGQTLRQNLFMADMSNTILYPVTPPANGANGGFIYNTSIIPVSAGL
FVNTWMYRQPASSRAYCQEPFGVAFGDTFENDRIAILIMAPDNLGSWSAV
APRNQTNIYLLVCSNATLCINPGFNRWGPAGSFIAPDALVDHSNSCFVNN
TFSVNISTSRISLAFLFKDGDLLIYHSGWLPTSNFEHGFSRGSHPMTYFM
SLPVGGNLPRAQFFQSIVRSNAIDKGDGMCTNFDVNLHVAHLINRDLLVS
YFNNGSVANAADCADSAAEELYCVTGSFDPPTGVYPLSRYRAQVAGFVRV
TQRGSYCTPPYSVLQDPPQPVVWRRYMLYDCVFDFTVVVDSLPTHQLQCY
GVSPRRLASMCYGSVTLDVMRINETHLNNLFNRVPDTFSLYNYALPDNFY
GCLHAFYLNSTAPYAVANRFPIKPGGRQSNSAFIDTVINAAHYSPFSYVY
GLAVITLKPAAGSKLVCPVANDTVVITDRCVQYNLYGYTGTGVLSKNTSL
VIPDGKVFTASSTGTIIGVSINSTTYSIMPCVTVPVSVGYHPNFERALLF
NGLSCSQRSRAVTEPVSVLWSASATAQDAFDTPSGCVVNVELRNTTIVNT
CAMPIGNSLCFINGSIATANADSLPRLQLVNYDPLYDNSTATPMTPVYWV
KVPTNFTLSATEEYIQTTAPKITIDCARYLCGDSSRCLNVLLHYGTFCND
INKALSRVSTILDSALLSLVKELSINTRDEVTTFSFDGDYNFTGLMGCLG
PNCGATTYRSAFSDLLYDKVRITDPGFMQSYQKCIDSQWGGSIRDLLCTQ
TYNGIAVLPPIVSPAMQALYTSLLVGAVASSGYTFGITSAGVIPFATQLQ
FRLNGIGVTTQVLVENQKLIASSFNNALVNIQKGFTETSIALSKMQDVIN
QHAAQLHTLVVQLGNSFGAISSSINEIFSRLEPPAANAEVDRLINGRMMV
LNTYVTQLLIQASEAKQNALAAQKISECVKAQSLRNDFCGNGTHVLSIP
QLAPNGVLFIHYAYTPTEYAFVQTSAGLCHNGTGYAPRQGMFVLPNNTNM
WHFTTMQFYNPVNISASNTQVLTSCSVNYTSVNYTVLEPSVPGDYDFQKE
FDKFYKNLSTIFNNTFNPNDFNFSTVDVTAQIKSLHDVVNQLNQSFIDLK KLNVYEKGGYIPEAPRDGQAYVRKDGEWVLLSTF

In some embodiments, the recombinant HKU9-CoV S ectodomain trimer comprises protomers comprising the ectodomain sequence of SEQ ID NO: 13. In some embodiments, the recombinant HKU9-CoV S ectodomain trimer comprises protomers comprising residues 15-1207 of SEQ ID NO: 13 or residues 15-1234 of SEQ ID NO: 32. In some embodiments, the recombinant HKU9-CoV S ectodomain trimer comprises protomers comprising an ectodomain sequence at least 90% identical to the ectodomain sequence of SEQ ID NO: 13, wherein the HKU9-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers. In some embodiments, the recombinant HKU9-CoV S ectodomain trimer comprises protomers comprising an amino acid sequence at least 90% identical to residues 15-1207 of SEQ ID NO: 13 or residues 15-1234 of SEQ ID NO: 32, wherein the HKU9-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers.

E. OC43-CoV

In some embodiments, the immunogen comprises a recombinant OC43-CoV S ectodomain trimer comprising protomers comprising one or more (such as two, for example two consecutive) proline substitutions at or near the boundary between a HR1 domain and a central helix domain that stabilize the S ectodomain trimer in the prefusion conformation. In some such embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the S ectodomain in the prefusion conformation are located between a position 15 amino acids N-terminal of a C-terminal residue of the HR1 and a position 5 amino acids C-terminal of a N-terminal residue of the central helix.

In some embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the OC43-CoV S ectodomain trimer in the prefusion conformation are located between residues 1062-1082 (such as between residues 1072-1082 or between residues 1077-1082) of the S ectodomain protomers in the trimer. In some embodiments, the OC43-CoV S ectodomain trimer is stabilized in the prefusion conformation by A1079P and L1080P substitutions ("2P") in the S ectodomain protomers in the trimer. The amino acid numbering for OC43-CoV S proteins is with reference to the OC43-CoV S sequence provided as SEQ ID NO: 10.

In some embodiments, the recombinant OC43-CoV S ectodomain trimer stabilized in the prefusion conformation comprises single-chain S ectodomain protomers comprising mutations to the S1/S2 and/or S2' protease cleavage sites to prevent protease cleavage at these sites.

In some embodiments, the protomers of the recombinant OC43-CoV S ectodomain trimer stabilized in the prefusion conformation by the one or more proline substitutions (such as A1079P and L1080P substitutions) comprise additional modifications for stabilization in the prefusion conformation.

With reference to the OC43-CoV S protein sequence provided as SEQ ID NO: 10, the ectodomain of the OC43-CoV S protein includes about residues 15-1301. Residues 1-14 are the signal peptide, which is removed during cellular processing. The S1/S2 cleavage site is located at about position 767/768. The S2' cleavage site is located at about position 912/913. The HR1 is located at about residues 1008-1076. The central helix is located at about residues 1081-1122. The HR2 is located at about 1257-1287. The C-terminal end of the S2 ectodomain is located at about residue 1301. In some embodiments, the protomers of the prefusion-stabilized OC43-CoV S ectodomain trimer can have a C-terminal residue (which can be linked to a trimerization domain, or a transmembrane domain, for example) of the C-terminal residue of the HR2 (e.g., position 1287), or the ectodomain (e.g., position 1301), or from one of positions 1287-1301. The position numbering of the S protein may vary between OC43-CoV stains, but the sequences can be aligned to determine relevant structural domains and cleavage sites. It will be appreciated that a few residues (such as up to 10) on the N and C-terminal ends of the ectodomain can be removed or modified in the disclosed immunogens without decreasing the utility of the S ectodomain trimer as an immunogen.

Exemplary OC43-CoV S protein sequences are provided below. The prefusion stabilizing substitutions disclosed herein (and other modifications, such as substitutions to generate a single chain) can be incorporated into OC43-CoV S protein sequences.

An exemplary sequence of OC43-CoV S protein (including the ectodomain and TM and CT domains) is provided as GenBank GI: 744516696, incorporated by reference herein. Another exemplary sequence of OC43-CoV S protein is provided as GenBank GI:549302, incorporated by reference herein):

TABLE-US-00016 (SEQ ID NO: 10) MFLILLISLPTAFAVIGDLKCPLDSRTGSLNNIDTGPPSISTATVDVTNG
LGTYYVLDRVYLNTTLFLNGYYPTSGSTYRNMALKGTDKLSTLWFKPPFL
SDFINGIFAKVKNTKVFKDGVMYSEFPAITIGSTFVNTSYSVVVQPRTIN
STQDGVNKLQGLLEVSVCQYNMCEYPHTICHPKLGNHFKELWHMDTGVVS
CLYKRNFTYDVNATYLYFHFYQEGGTFYAYFTDTGVVTKFLFNVYLGMAL
SHYYVMPLTCISRRDIGFTLEYWVTPLTSRQYLLAFNQDGIIFNAVDCMS
DFMSEIKCKTQSIAPPTGVYELNGYTVQPIADVYRRKPDLPNCNIEAWLN
DKSVPSPLNWERKTFSNCNFNMSSLMSFIQADSFTCNNIDAAKIYGMCFS
SITIDKFAIPNGRKVDLQLGNLGYLQSFNYRIDTTATSCQLYYNLPAANV
SVSRFNPSTWNKRFGFIENSVFKPQPAGVLTNHDVVYAQHCFKAPKNFCP
CKLNSSLCVGSGPGKNNGIGTCPAGTNYLTCHNLCNPDPITFTGPYKCPQ
TKSLVGIGEHCSGLAVKSDYCGGNPCTCQPQAFLGWSADSCLQGDKCNIF
ANLILHDVNSGLTCSTDLQKANTDIKLGVCVNYDLYGISGQGIFVEVNAT
YYNSWQNLLYDSNGNLYGFRDYITNRTFMIRSCYSGRVSAAFHANSSEPA
LLFRNIKCNYVFNNSLIRQLQPINYFDSYLGCVVNAYNSTAISVQTCDLT
VGSGYCVDYSKNRRSRRAITTGYRFTNFEPFTVNSVNDSLEPVGGLYEIQ
IPSEFTIGNMEEFIQTSSPKVTIDCAAFVCGDYAACKSQLVEYGSFCDNI
NAILTEVNELLDTTQLQVANSLMNGVTLSTKLKDGVNFNVDDINFSSVLG
CLGSECSKASSRSAIEDLLFDKVKLSDVGFVAAYNNCTGGAEIRDLICVQ
SYKGIKVLPPLLSENQISGYTLAATSASLFPPWTAAAGVPFYLNVQYRIN
GLGVTMDVLSQNQKLIANAFNNALDAIQEGFDATNSALVKIQAVVNANAE
ALNNLLQQLSNRFGAISSSLQEILSRLDALEAEAQIDRLINGRLTALNAY
VSQQLSDSTLVKFSAAQAMEKVNECVKSQSSRINFCGNGNHIISLVQNAP
YGLYFIHFSYVPTKYVTAKVSPGLCIAGDRGIAPKSGYFVNVNNTWMYTG
SGYYYPEPITENNVVVMSTCAVNYTKAPYVMLNTSTPNLPDFREELDQWF

KNQTSVAPDLSLDYINVTFLDLQVEMNRLQEAIKVLNQSYINLKDIGTYE
YYVKWPWYVWLLIGLAGVAMLVLLFFICCCTGCGTSCFKKCGGCCDDYTG YQELVIKTSHDD

An exemplary sequence of OC43-CoV S ectodomain including a double proline substitution for stabilization in the prefusion conformation is provided as SEQ ID NO: 11, which also includes mutations to eliminate the S1/S2 cleavage site:

TABLE-US-00017 MFLILLISLPTAFAVIGDLKCPLDSRTGSLNNIDTGPPSISTATVDVTNG
LGTYYVLDRVYLNTTLFLNGYYPTSGSTYRNMALKGTDKLSTLWFKPPFL
SDFINGIFAKVKNTKVFKDGVMYSEFPAITIGSTFVNTSYSVVVQPRTIN
STQDGVNKLQGLLEVSVCQYNMCEYPHTICHPKLGNHFKELWHMDTGVVS
CLYKRNFTYDVNATYLYFHFYQEGGTFYAYFTDTGVVTKFLFNVYLGMAL
SHYYVMPLTCISRRDIGFTLEYWVTPLTSRQYLLAFNQDGIIFNAVDCMS
DFMSEIKCKTQSIAPPTGVYELNGYTVQPIADVYRRKPDLPNCNIEAWLN
DKSVPSPLNWERKTFSNCNFNMSSLMSFIQADSFTCNNIDAAKIYGMCFS
SITIDKFAIPNGRKVDLQLGNLGYLQSFNYRIDTTATSCQLYYNLPAANV
SVSRFNPSTWNKRFGFIENSVFKPQPAGVLTNHDVVYAQHCFKAPKNFCP
CKLNSSLCVGSGPGKNNGIGTCPAGTNYLTCHNLCNPDPITFTGPYKCPQ
TKSLVGIGEHCSGLAVKSDYCGGNPCTCQPQAFLGWSADSCLQGDKCNIF
ANLILHDVNSGLTCSTDLQKANTDIKLGVCVNYDLYGISGQGIFVEVNAT
YYNSWQNLLYDSNGNLYGFRDYITNRTFMIRSCYSGRVSAAFHANSSEPA
LLFRNIKCNYVFNNSLIRQLQPINYFDSYLGCVVNAYNSTAISVQTCDLT
VGSGYCVDYSKNGGSGSAITTGYRFTNFEPFTVNSVNDSLEPVGGLYEIQ
IPSEFTIGNMEEFIQTSSPKVTIDCAAFVCGDYAACKSQLVEYGSFCDNI
NAILTEVNELLDTTQLQVANSLMNGVTLSTKLKDGVNFNVDDINFSSVLG
CLGSECSKASSRSAIEDLLFDKVKLSDVGFVAAYNNCTGGAEIRDLICVQ
SYKGIKVLPPLLSENQISGYTLAATSASLFPPWTAAAGVPFYLNVQYRIN
GLGVTMDVLSQNQKLIANAFNNALDAIQEGFDATNSALVKIQAVVNANAE
ALNNLLQQLSNRFGAISSSLQEILSRLDPPEAEAQIDRLINGRLTALNAY
VSQQLSDSTLVKFSAAQAMEKVNECVKSQSSRINFCGNGNHIISLVQNAP
YGLYFIHFSYVPTKYVTAKVSPGLCIAGDRGIAPKSGYFVNVNNTWMYTG
SGYYYPEPITENNVVVMSTCAVNYTKAPYVMLNTSTPNLPDFREELDQWF KNQTSVAPDLSLDYINVTFLDLQVEMNRLQEAIKVLN

A C-terminal trimerization domain can be added to the protomers of the OC43-CoV S ectodomains trimer to promote trimerization of the ectodomain.

An exemplary sequence of OC43-CoV S ectodomain including a double proline substitution for stabilization in the prefusion conformation, mutations to eliminate the S1/S2 cleavage site, and a T4 fibritin trimerization domain is provided as SEQ ID NO: 33:

TABLE-US-00018 MFLILLISLPTAFAVIGDLKCPLDSRTGSLNNIDTGPPSISTATVDVTNG
LGTYYVLDRVYLNTTLFLNGYYPTSGSTYRNMALKGTDKLSTLWFKPPFL
SDFINGIFAKVKNTKVFKDGVMYSEFPAITIGSTFVNTSYSVVVQPRTIN
STQDGVNKLQGLLEVSVCQYNMCEYPHTICHPKLGNHFKELWHMDTGVVS
CLYKRNFTYDVNATYLYFHFYQEGGTFYAYFTDTGVVTKFLFNVYLGMAL
SHYYVMPLTCISRRDIGFTLEYWVTPLTSRQYLLAFNQDGIIFNAVDCMS
DFMSEIKCKTQSIAPPTGVYELNGYTVQPIADVYRRKPDLPNCNIEAWLN
DKSVPSPLNWERKTFSNCNFNMSSLMSFIQADSFTCNNIDAAKIYGMCFS
SITIDKFAIPNGRKVDLQLGNLGYLQSFNYRIDTTATSCQLYYNLPAANV
SVSRFNPSTWNKRFGFIENSVFKPQPAGVLTNHDVVYAQHCFKAPKNFCP
CKLNSSLCVGSGPGKNNGIGTCPAGTNYLTCHNLCNPDPITFTGPYKCPQ
TKSLVGIGEHCSGLAVKSDYCGGNPCTCQPQAFLGWSADSCLQGDKCNIF
ANLILHDVNSGLTCSTDLQKANTDIKLGVCVNYDLYGISGQGIFVEVNAT
YYNSWQNLLYDSNGNLYGFRDYITNRTFMIRSCYSGRVSAAFHANSSEPA
LLFRNIKCNYVFNNSLIRQLQPINYFDSYLGCVVNAYNSTAISVQTCDLT
VGSGYCVDYSKNGGSGSAITTGYRFTNFEPFTVNSVNDSLEPVGGLYEIQ
IPSEFTIGNMEEFIQTSSPKVTIDCAAFVCGDYAACKSQLVEYGSFCDNI
NAILTEVNELLDTTQLQVANSLMNGVTLSTKLKDGVNFNVDDINFSSVLG
CLGSECSKASSRSAIEDLLFDKVKLSDVGFVAAYNNCTGGAEIRDLICVQ
SYKGIKVLPPLLSENQISGYTLAATSASLFPPWTAAAGVPFYLNVQYRIN
GLGVTMDVLSQNQKLIANAFNNALDAIQEGFDATNSALVKIQAVVNANAE
ALNNLLQQLSNRFGAISSSLQEILSRLDPPEAEAQIDRLINGRLTALNAY
VSQQLSDSTLVKFSAAQAMEKVNECVKSQSSRINFCGNGNHIISLVQNAP
YGLYFIHFSYVPTKYVTAKVSPGLCIAGDRGIAPKSGYFVNVNNTWMYTG
SGYYYPEPITENNVVVMSTCAVNYTKAPYVMLNTSTPNLPDFREELDQWF
KNQTSVAPDLSLDYINVTFLDLQVEMNRLQEAIKVLNGGYIPEAPRDGQA YVRKDGEWVLLSTF

In some embodiments, the recombinant OC43-CoV S ectodomain trimer comprises protomers comprising the ectodomain sequence of SEQ ID NO: 11. In some embodiments, the recombinant OC43-CoV S ectodomain trimer comprises protomers comprising residues 15-1287 of SEQ ID NO: 11 or residues 15-1314 of SEQ ID NO: 33. In some embodiments, the recombinant OC43-CoV S ectodomain

trimer comprises protomers comprising an ectodomain sequence at least 90% identical to the ectodomain sequence of SEQ ID NO: 11 or residues 15-1314 of SEQ ID NO: 33, wherein the OC43-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers. In some embodiments, the recombinant OC43-CoV S ectodomain trimer comprises protomers comprising an amino acid sequence at least 90% identical to residues 15-1287 of SEQ ID NO: 11, wherein the OC43-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers.

F. WIV1-CoV

In some embodiments, the immunogen comprises a recombinant WIV1-CoV S ectodomain trimer comprising protomers comprising one or more (such as two, for example two consecutive) proline substitutions at or near the boundary between a HR1 domain and a central helix domain that stabilize the S ectodomain trimer in the prefusion conformation. In some such embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the S ectodomain in the prefusion conformation are located between a position 15 amino acids N-terminal of a C-terminal residue of the HR1 and a position 5 amino acids C-terminal of a N-terminal residue of the central helix.

In some embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the WIV1-CoV S ectodomain trimer in the prefusion conformation are located between residues 952 to 972 (such as between residues 962 to 972 or between residues 967 to 972) of the S ectodomain protomers in the trimer. In some embodiments, the WIV1-CoV S ectodomain trimer is stabilized in the prefusion conformation by K969P and V970P substitutions ("2P") in the S ectodomain protomers in the trimer. The amino acid numbering for WIV1-CoV S proteins is with reference to the WIV1-CoV S sequence provided as SEQ ID NO: 14.

In some embodiments, the recombinant WIV1-CoV S ectodomain trimer stabilized in the prefusion conformation comprises single-chain S ectodomain protomers comprising mutations to the S1/S2 and/or S2' protease cleavage sites to prevent protease cleavage at these sites.

In some embodiments, the protomers of the recombinant WIV1-CoV S ectodomain trimer stabilized in the prefusion conformation by the one or more proline substitutions (such as K969P and V970P substitutions) comprises additional modifications for stabilization in the prefusion conformation.

With reference to the WIV1-CoV S protein sequence provided as SEQ ID NO: 14, the ectodomain of the WIV1-CoV S protein includes about residues 16-1191. Residues 1-15 are the signal peptide, which is removed during cellular processing. The S1/S2 cleavage site is located at about position 668/669. The S2' cleavage site is located at about position 798/799. The HR1 is located at about residues 898-996. The central helix is located at about residues 971-1012. The HR2 is located at about 1146-1177. The C-terminal end of the S2 ectodomain is located at about residue 1191. In some embodiments, the protomers of the prefusion-stabilized WIV1-CoV S ectodomain trimer can have a C-terminal residue (which can be linked to a trimerization domain, or a transmembrane domain, for example) of the C-terminal residue of the HR2 (e.g., position 1177), or the ectodomain (e.g., position 1191), or from one of positions 1177-1191. The position numbering of the S protein may vary between WIV1-CoV stains, but the sequences can be aligned to determine relevant structural domains and cleavage sites. It will be appreciated that a few residues (such as up to 10) on the N and C-terminal ends of the ectodomain can be removed or modified in the disclosed immunogens without decreasing the utility of the S ectodomain trimer as an immunogen.

Exemplary WIV1-CoV S protein sequences are provided below. The prefusion stabilizing substitutions disclosed herein (and other modifications, such as substitutions to generate a single chain) can be incorporated into WIV1-CoV S protein sequences.

An exemplary sequence of WIV1-CoV S protein (including the ectodomain and TM and CT domains) is provided as SEQ ID NO: 14 (GenBank GI: 556015140, incorporated by reference herein):

TABLE-US-00019 MKLLVLVFATLVSSYTIEKCLDFDDRTPPANTQFLSSHRGVYYPDDIFRS
NVLHLVQDHFLPFDSNVTRFITFGLNFDNPIIPFKDGIYFAATEKSNVIR
GWVFGSTMNNKSQSVIIMNNSTNLVIRACNFELCDNPFFVVLKSNNTQIP
SYIFNNAFNCTFEYVSKDFNLDLGEKPGNFKDLREFVFRNKDGFLHVYSG
YQPISAASGLPTGFNALKPIFKLPLGINITNFRTLLTAFPPRPDYWGTSA
AAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAELKCSVKSFEIDKGI
YQTSNFRVAPSKEVVRFPNITNLCPFGEVFNATTFPSVYAWERKRISNCV
ADYSVLYNSTSFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAP
GQTGVIADYNYKLPDDFTGCVLAWNTRNIDATQTGNYNYKYRSLRHGKLR
PFERDISNVPFSPDGKPCTPPAFNCYWPLNDYGFYITNGIGYQPYRVVVL
SFELLNAPATVCGPKLSTDLIKNQCVNFNFNGLTGTGVLTPSSKRFQPFQ
QFGRDVSDFTDSVRDPKTSEILDISPCSFGGVSVITPGTNTSSEVAVLYQ
DVNCTDVPVAIHADQLTPSWRVHSTGNNVFQTQAGCLIGAEHVDTSYECD
IPIGAGICASYHTVSSLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTN
FSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRAL

SGIAVEQDRNTREVFAQVKQMYKTPTLKDFGGFNFSQILPDPLKPTKRSF
IEDLLFNKVTLADAGFMKQYGECLGDINARDLICAQKFNGLTVLPPLLTD
DMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLY
ENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLS
SNFGAISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAE
IRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTY
VPSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITT
DNTFVSGSCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLG
DISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYVW
LGFIAGLIAIVMVTILLCCMTSCCSCLKGACSCGSCCKFDEDDSEPVLKG VKLHYT

An exemplary sequence of WIV1-CoV S protein including a double proline substitution for stabilization in the prefusion conformation is provided as SEQ ID NO: 15:

TABLE-US-00020 MKLLVLVFATLVSSYTIEKCLDFDDRTPPANTQFLSSHRGVYYPDDIFRS
NVLHLVQDHFLPFDSNVTRFITFGLNFDNPIIPFKDGIYFAATEKSNVIR
GWVFGSTMNNKSQSVIIMNNSTNLVIRACNFELCDNPFFVVLKSNNTQIP
SYIFNNAFNCTFEYVSKDFNLDLGEKPGNFKDLREFVFRNKDGFLHVYSG
YQPISAASGLPTGFNALKPIFKLPLGINITNFRTLLTAFPPRPDYWGTSA
AAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAELKCSVKSFEIDKGI
YQTSNFRVAPSKEVVRFPNITNLCPFGEVFNATTFPSVYAWERKRISNCV
ADYSVLYNSTSFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAP
GQTGVIADYNYKLPDDFTGCVLAWNTRNIDATQTGNYNYKYRSLRHGKLR
PFERDISNVPFSPDGKPCTPPAFNCYWPLNDYGFYITNGIGYQPYRVVVL
SFELLNAPATVCGPKLSTDLIKNQCVNFNFNGLTGTGVLTPSSKRFQPFQ
QFGRDVSDFTDSVRDPKTSEILDISPCSFGGVSVITPGTNTSSEVAVLYQ
DVNCTDVPVAIHADQLTPSWRVHSTGNNVFQTQAGCLIGAEHVDTSYECD
IPIGAGICASYHTVSSLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTN
FSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRAL
SGIAVEQDRNTREVFAQVKQMYKTPTLKDFGGFNFSQILPDPLKPTKRSF
IEDLLFNKVTLADAGFMKQYGECLGDINARDLICAQKFNGLTVLPPLLTD
DMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLY
ENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLS
SNFGAISSVLNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAE
IRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTY
VPSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITT
DNTFVSGSCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLG DISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQ

A C-terminal trimerization domain can be added to the protomers of the WIV1-CoV S ectodomains trimer to promote trimerization of the ectodomain.

An exemplary sequence of WIV1-CoV S protein including a double proline substitution for stabilization in the prefusion conformation, and a T4 fibritin trimerization domain is provided as SEQ ID NO: 34:

TABLE-US-00021 MKLLVLVFATLVSSYTIEKCLDFDDRTPPANTQFLSSHRGVYYPDDIFRS
NVLHLVQDHFLPFDSNVTRFITFGLNFDNPIIPFKDGIYFAATEKSNVIR
GWVFGSTMNNKSQSVIIMNNSTNLVIRACNFELCDNPFFVVLKSNNTQIP
SYIFNNAFNCTFEYVSKDFNLDLGEKPGNFKDLREFVFRNKDGFLHVYSG
YQPISAASGLPTGFNALKPIFKLPLGINITNFRTLLTAFPPRPDYWGTSA
AAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAELKCSVKSFEIDKGI
YQTSNFRVAPSKEVVRFPNITNLCPFGEVFNATTFPSVYAWERKRISNCV
ADYSVLYNSTSFSTFKCYGVSATKLNDLCFSNVYADSFVVKGDDVRQIAP
GQTGVIADYNYKLPDDFTGCVLAWNTRNIDATQTGNYNYKYRSLRHGKLR
PFERDISNVPFSPDGKPCTPPAFNCYWPLNDYGFYITNGIGYQPYRVVVL
SFELLNAPATVCGPKLSTDLIKNQCVNFNFNGLTGTGVLTPSSKRFQPFQ
QFGRDVSDFTDSVRDPKTSEILDISPCSFGGVSVITPGTNTSSEVAVLYQ
DVNCTDVPVAIHADQLTPSWRVHSTGNNVFQTQAGCLIGAEHVDTSYECD
IPIGAGICASYHTVSSLRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTN
FSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRAL
SGIAVEQDRNTREVFAQVKQMYKTPTLKDFGGFNFSQILPDPLKPTKRSF
IEDLLFNKVTLADAGFMKQYGECLGDINARDLICAQKFNGLTVLPPLLTD
DMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLY
ENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLS
SNFGAISSVLNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAE
IRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTY
VPSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITT
DNTFVSGSCDVVIGIINNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLG
DISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQGGYIPEAPR DGQAYVRKDGEWVLLSTF

In some embodiments, the recombinant WIV1-CoV S ectodomain trimer comprises protomers comprising the ectodomain sequence of SEQ ID NO: 15. In some embodiments, the recombinant WIV1-CoV S ectodomain trimer comprises protomers comprising residues 16-1191 of SEQ ID NO: 15 or residues 16-1218 of SEQ ID NO: 34. In some embodiments, the recombinant WIV1-CoV S ectodomain trimer comprises protomers comprising an ectodomain sequence at least 90% identical to the ectodomain sequence of SEQ ID NO: 15, wherein the WIV1-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers. In some embodiments, the recombinant WIV1-CoV S ectodomain trimer comprises protomers comprising an amino acid sequence at least 90% identical to residues 16-1191 of SEQ ID NO: 15 or residues 16-1218 of SEQ ID NO: 34, wherein the WIV1-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers.

G. MHV-CoV

In some embodiments, the immunogen comprises a recombinant MHV-CoV S ectodomain trimer comprising protomers comprising one or more (such as two, for example two consecutive) proline substitutions at or near the boundary between a HR1 domain and a central helix domain that stabilize the S ectodomain trimer in the prefusion conformation. In some such embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the S ectodomain in the prefusion conformation are located between a position 15 amino acids N-terminal of a C-terminal residue of the HR1 and a position 5 amino acids C-terminal of a N-terminal residue of the central helix.

In some embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the MHV-CoV S ectodomain trimer in the prefusion conformation are located between residues 852 to 872 (such as between residues 862 to 872 or between residues 867 to 872) of the S ectodomain protomers in the trimer. In some embodiments, the MHV-CoV S ectodomain trimer is stabilized in the prefusion conformation by I869P and I870P substitutions ("2P") in the S ectodomain protomers in the trimer. The amino acid numbering for MHV-CoV S proteins is with reference to the MHV-CoV S sequence provided as SEQ ID NO: 16.

In some embodiments, the recombinant MHV-CoV S ectodomain trimer stabilized in the prefusion conformation comprises single-chain S ectodomain protomers comprising mutations to the S1/S2 and/or S2' protease cleavage sites to prevent protease cleavage at these sites.

In some embodiments, the protomers of the recombinant MHV-CoV S ectodomain trimer stabilized in the prefusion conformation by the one or more proline substitutions (such as I869P and 1870P substitutions) comprises additional modifications for stabilization in the prefusion conformation.

With reference to the MHV-CoV S protein sequence provided as SEQ ID NO: 16, the ectodomain of the MHV-CoV S protein includes about residues 15-1297. Residues 1-14 are the signal peptide, which is removed during cellular processing. The S1/S2 cleavage site is located at about position 757/758. The S2' cleavage site is located at about position 906/907. The HR1 is located at about residues 1002-1070. The central helix is located at about residues 1075-1116. The HR2 is located at about 1252-1283. The C-terminal end of the S2 ectodomain is located at about residue 1297. In some embodiments, the protomers of the prefusion-stabilized MHV-CoV S ectodomain trimer can have a C-terminal residue (which can be linked to a trimerization domain, or a transmembrane domain, for example) of the C-terminal residue of the HR2 (e.g., position 1283), or the ectodomain (e.g., position 1297), or from one of positions 1283-1297. The position numbering of the S protein may vary between MHV-CoV stains, but the sequences can be aligned to determine relevant structural domains and cleavage sites. It will be appreciated that a few residues (such as up to 10) on the N and C-terminal ends of the ectodomain can be removed or modified in the disclosed immunogens without decreasing the utility of the S ectodomain trimer as an immunogen.

Exemplary MHV-CoV S protein sequences are provided below. The prefusion stabilizing substitutions disclosed herein (and other modifications, such as substitutions to generate a single chain) can be incorporated into MHV-CoV S protein sequences.

An exemplary sequence of MHV-CoV S protein (including the ectodomain and TM and CT domains) is provided as SEQ ID NO: 16 (GenBank GI:328496819, incorporated by reference herein). Another exemplary MHV-CoV sequence is provided as GenBank GI:81971726, incorporated by reference herein:

TABLE-US-00022 MLSVFILFLPSCLGYIGDFRCINLVNTDTSNASAPSVSTEVVDVSKGIGT
YYVLDRVYLNATLLLTGYYPVDGSNYRNLALTGTNTLSLNWYKPPFLSEF
NDGIFAKVKNLKASLPKDSTSYFPTIVIGSNFVTTSYTVVLEPYNGIIMA
SICQYTICLLPYTDCKPNTGGNKLIGFWHIDLKSPVCILKRNFTFNVNAD
WLYFHFYQQGGTFYAYYADAGSATTFLFSSYIGDVLTQYFVLPFVCTPTT
TGVFSPQYWVTPLVKRQYLFNFNQKGTITSAVDCASSYTSEIKCKTQSMN
PNTGVYDLSGYTVQPVGLVYRRVRNLPDCRIEDWLAAKTVPSPLNWERKT
FQNCNFNLSSLLRLVQAGSLSCSNIDAAKVYGMCFGSMSIDKFAIPNSRR
VDLQLGNSGFLQSFNYKIDTRATSCQLYYSLAQSNVTVNNHNPSSWNRRY

GFNDVATFGRGKHDVAYAEACFTVGASYCPCANPSIVSPCTTGKPKFANC
PTGTTNRECNVLALGSNLFKCDCTCNPSPLTTYDLRCLQGRSMLGVGDHC
EGLGVLEDKCGGSNTCNCSADAFVGWAKDSCLSNGRCHIFSNLMLNGINS
GTTCSTDLQLPNTEVVTGICVKYDLYGITGQGVFKEVKADYYNSWQNLLY
DVNGNLNGFRDIVTNKTYLTRSCYSGRVSAAYHQDAPEPALLYRNLKCDY
VFNNNIFREETPLNYFDSYLGCVVNADNSTEQAVDACDLRMGSGLCVNYS
TAHRARTSVSTGYKLTTFEPFTVSIVNDSVESVGGLYEMQIPTNFTIASH
QEFIQTRAPKVTIDCAAFVCGDYTTCRQQLVEYGSFCDNINAILGEVNNL
IDTMQLQVASALIQGVTLSSRLADGISGQIDDINFSPLLGCLGSQCSEGT
MAAQGRSTVEDLLFDKVKLSDVGFVEAYNNCTGGQEVRDLLCVQSFNGIK
VLPPVLSENQVSGYTAGATASSMFPPWSAAAGVPFSLSVQYRINGLGVTM
NVLSENQKMIASAFNNAIGAIQEGFDATNSALAKIQSVVNANAEALNNLL
NQLSNRFGAISASLQEILSRLDALEAQAQIDRLINGRLTALNAYVSKQLS
DMTLIKVSAAQAIEKVNECVKSQSPRINFCGNGNHILSLVQNAPYGLYFL
HFSYVPTSFTTANVSPGLCISGDRGLAPKAGYFVQDDGEWKFTGSNYYYP
EPITDKNSVVMSSCAVNYTKAPEVFLNTSISNLPDFKEELDKWFKNQTSV
APDLSLDFEKLNVTFLDLSDEMNRIQEAIKKLNESYINLKEIGTYEMYVK
WPWYVWLLIGLAGVAVCVLLFFICCCTGCGSCCFKKCGNCCDEYGGHQDS IVIHNISSHED

An exemplary sequence of MHV-CoV S ectodomain including a double proline substitution for stabilization in the prefusion conformation is provided as SEQ ID NO: 17:

TABLE-US-00023 MLSVFILFLPSCLGYIGDFRCINLVNTDTSNASAPSVSTEVVDVSKGIGT
YYVLDRVYLNATLLLTGYYPVDGSNYRNLALTGTNTLSLNWYKPPFLSEF
NDGIFAKVKNLKASLPKDSTSYFPTIVIGSNFVTTSYTVVLEPYNGIIMA
SICQYTICLLPYTDCKPNTGGNKLIGFWHIDLKSPVCILKRNFTFNVNAD
WLYFHFYQQGGTFYAYYADAGSATTFLFSSYIGDVLTQYFVLPFVCTPTT
TGVFSPQYWVTPLVKRQYLFNFNQKGTITSAVDCASSYTSEIKCKTQSMN
PNTGVYDLSGYTVQPVGLVYRRVRNLPDCRIEDWLAAKTVPSPLNWERKT
FQNCNFNLSSLLRLVQAGSLSCSNIDAAKVYGMCFGSMSIDKFAIPNSRR
VDLQLGNSGFLQSFNYKIDTRATSCQLYYSLAQSNVTVNNHNPSSWNRRY
GFNDVATFGRGKHDVAYAEACFTVGASYCPCANPSIVSPCTTGKPKFANC
PTGTTNRECNVLALGSNLFKCDCTCNPSPLTTYDLRCLQGRSMLGVGDHC
EGLGVLEDKCGGSNTCNCSADAFVGWAKDSCLSNGRCHIFSNLMLNGINS
GTTCSTDLQLPNTEVVTGICVKYDLYGITGQGVFKEVKADYYNSWQNLLY
DVNGNLNGFRDIVTNKTYLTRSCYSGRVSAAYHQDAPEPALLYRNLKCDY
VFNNNIFREETPLNYFDSYLGCVVNADNSTEQAVDACDLRMGSGLCVNYS
TAHRARTSVSTGYKLTTFEPFTVSIVNDSVESVGGLYEMQIPTNFTIASH
QEFIQTRAPKVTIDCAAFVCGDYTTCRQQLVEYGSFCDNINAILGEVNNL
IDTMQLQVASALIQGVTLSSRLADGISGQIDDINFSPLLGCLGSQCSEGT
MAAQGRSTVEDLLFDKVKLSDVGFVEAYNNCTGGQEVRDLLCVQSFNGIK
VLPPVLSENQVSGYTAGATASSMFPPWSAAAGVPFSLSVQYRINGLGVTM
NVLSENQKMIASAFNNAIGAIQEGFDATNSALAKIQSVVNANAEALNNLL
NQLSNRFGAISASLQEILSRLDPPEAQAQIDRLINGRLTALNAYVSKQLS
DMTLIKVSAAQAIEKVNECVKSQSPRINFCGNGNHILSLVQNAPYGLYFL
HFSYVPTSFTTANVSPGLCISGDRGLAPKAGYFVQDDGEWKFTGSNYYYP
EPITDKNSVVMSSCAVNYTKAPEVFLNTSISNLPDFKEELDKWFKNQTSV
APDLSLDFEKLNVTFLDLSDEMNRIQEAIKKLNESYINLKEIGTYEM

A C-terminal trimerization domain can be added to the protomers of the MHV-CoV S ectodomains trimer to promote trimerization of the ectodomain.

An exemplary sequence of MHV-CoV S ectodomain including a double proline substitution for stabilization in the prefusion conformation, and a T4 fibritin trimerization domain is provided as SEQ ID NO: 35:

TABLE-US-00024 MLSVFILFLPSCLGYIGDFRCINLVNTDTSNASAPSVSTEVVDVSKGIGT
YYVLDRVYLNATLLLTGYYPVDGSNYRNLALTGTNTLSLNWYKPPFLSEF
NDGIFAKVKNLKASLPKDSTSYFPTIVIGSNFVTTSYTVVLEPYNGIIMA
SICQYTICLLPYTDCKPNTGGNKLIGFWHIDLKSPVCILKRNFTFNVNAD
WLYFHFYQQGGTFYAYYADAGSATTFLFSSYIGDVLTQYFVLPFVCTPTT
TGVFSPQYWVTPLVKRQYLFNFNQKGTITSAVDCASSYTSEIKCKTQSMN
PNTGVYDLSGYTVQPVGLVYRRVRNLPDCRIEDWLAAKTVPSPLNWERKT
FQNCNFNLSSLLRLVQAGSLSCSNIDAAKVYGMCFGSMSIDKFAIPNSRR
VDLQLGNSGFLQSFNYKIDTRATSCQLYYSLAQSNVTVNNHNPSSWNRRY
GFNDVATFGRGKHDVAYAEACFTVGASYCPCANPSIVSPCTTGKPKFANC
PTGTTNRECNVLALGSNLFKCDCTCNPSPLTTYDLRCLQGRSMLGVGDHC
EGLGVLEDKCGGSNTCNCSADAFVGWAKDSCLSNGRCHIFSNLMLNGINS
GTTCSTDLQLPNTEVVTGICVKYDLYGITGQGVFKEVKADYYNSWQNLLY

DVNGNLNGFRDIVTNKTYLTRSCYSGRVSAAYHQDAPEPALLYRNLKCDY
VFNNNIFREETPLNYFDSYLGCVVNADNSTEQAVDACDLRMGSGLCVNYS
TAHRARTSVSTGYKLTTFEPFTVSIVNDSVESVGGLYEMQIPTNFTIASH
QEFIQTRAPKVTIDCAAFVCGDYTTCRQQLVEYGSFCDNINAILGEVNNL
IDTMQLQVASALIQGVTLSSRLADGISGQIDDINFSPLLGCLGSQCSEGT
MAAQGRSTVEDLLFDKVKLSDVGFVEAYNNCTGGQEVRDLLCVQSFNGIK
VLPPVLSENQVSGYTAGATASSMFPPWSAAAGVPFSLSVQYRINGLGVTM
NVLSENQKMIASAFNNAIGAIQEGFDATNSALAKIQSVVNANAEALNNLL
NQLSNRFGAISASLQEILSRLDPPEAQAQIDRLINGRLTALNAYVSKQLS
DMTLIKVSAAQAIEKVNECVKSQSPRINFCGNGNHILSLVQNAPYGLYFL
HFSYVPTSFTTANVSPGLCISGDRGLAPKAGYFVQDDGEWKFTGSNYYYP
EPITDKNSVVMSSCAVNYTKAPEVFLNTSISNLPDFKEELDKWFKNQTSV
APDLSLDFEKLNVTFLDLSDEMNRIQEAIKKLNESYINLKEIGTYEMGGY IPEAPRDGQAYVRKDGEWVLLSTF

In some embodiments, the recombinant MHV-CoV S ectodomain trimer comprises protomers comprising the ectodomain sequence of SEQ ID NO: 17. In some embodiments, the recombinant MHV-CoV S ectodomain trimer comprises protomers comprising residues 15-1297 of SEQ ID NO: 17 or residues 15-1324 of SEQ ID NO: 35. In some embodiments, the recombinant MHV-CoV S ectodomain trimer comprises protomers comprising an ectodomain sequence at least 90% identical to the ectodomain sequence of SEQ ID NO: 17, wherein the MHV-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers. In some embodiments, the recombinant MHV-CoV S ectodomain trimer comprises protomers comprising an amino acid sequence at least 90% identical to residues 15-1297 of SEQ ID NO: 17 or residues 15-1324 of SEQ ID NO: 35, wherein the MHV-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers.

H. NL63-CoV

In some embodiments, the immunogen comprises a recombinant NL63-CoV S ectodomain trimer comprising protomers comprising one or more (such as two, for example two consecutive) proline substitutions at or near the boundary between a HR1 domain and a central helix domain that stabilize the S ectodomain trimer in the prefusion conformation. In some such embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the S ectodomain in the prefusion conformation are located between a position 15 amino acids N-terminal of a C-terminal residue of the HR1 and a position 5 amino acids C-terminal of a N-terminal residue of the central helix.

In some embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the NL63-CoV S ectodomain trimer in the prefusion conformation are located between residues 1035 to 1055 (such as between residues 1045 to 1055 or between residues 1050 to 1055) of the S ectodomain protomers in the trimer. In some embodiments, the NL63-CoV S ectodomain trimer is stabilized in the prefusion conformation by S1052P and I1053P substitutions ("2P") in the S ectodomain protomers in the trimer. The amino acid numbering for NL63-CoV S proteins is with reference to the NL63-CoV S sequence provided as SEQ ID NO: 18.

In some embodiments, the recombinant NL63-CoV S ectodomain trimer stabilized in the prefusion conformation comprises single-chain S ectodomain protomers comprising mutations to the S1/S2 and/or S2' protease cleavage sites to prevent protease cleavage at these sites.

In some embodiments, the protomers of the recombinant NL63-CoV S ectodomain trimer stabilized in the prefusion conformation by the one or more proline substitutions (such as S1052P and I1053P substitutions) comprises additional modifications for stabilization in the prefusion conformation.

With reference to the NL63-CoV S protein sequence provided as SEQ ID NO: 18, the ectodomain of the NL63-CoV S protein includes about residues 16-1291. Residues 1-15 are the signal peptide, which is removed during cellular processing. The S1/S2 cleavage site is located at about position 748/749. The S2' cleavage site is located at about position 870/871. The HR1 is located at about residues 967-1049. The central helix is located at about residues 1054-1095. The HR2 is located at about 1246-1272. The C-terminal end of the S2 ectodomain is located at about residue 1291. In some embodiments, the protomers of the prefusion-stabilized NL63-CoV S ectodomain trimer can have a C-terminal residue (which can be linked to a trimerization domain, or a transmembrane domain, for example) of the C-terminal residue of the HR2 (e.g., position 1277), or the ectodomain (e.g., position 1291), or from one of positions 1277-1291. The position numbering of the S protein may vary between NL63-CoV stains, but the sequences can be aligned to determine relevant structural domains and cleavage sites. It will be appreciated that a few residues (such as up to 10) on the N and C-terminal ends of the ectodomain can be removed or modified in the disclosed immunogens without decreasing the utility of the S ectodomain trimer as an immunogen.

Exemplary NL63-CoV S protein sequences are provided below. The prefusion stabilizing substitutions disclosed herein (and other modifications, such as substitutions to generate a single chain) can be incorporated into NL63-CoV S protein sequences.

An exemplary sequence of NL63-CoV S protein (including the ectodomain and TM and CT domains) is provided as SEQ ID NO: 18 (GenBank GI: 71153773, incorporated by reference herein):

TABLE-US-00025 MKLFLILLVLPLASCFFTCNSNANLSMLQLGVPDNSSTIVTGLLPTHWFC
ANQSTSVYSANGFFYIDVGNHRSAFALHTGYYDANQYYIYVTNEIGLNAS
VTLKICKFSRNTTFDFLSNASSSFDCIVNLLFTEQLGAPLGITISGETVR
LHLYNVTRTFYVPAAYKLTKLSVKCYFNYSCVFSVVNATVTVNVTTHNGR
VVNYTVCDDCNGYTDNIFSVQQDGRIPNGFPFNNWFLLTNGSTLVDGVSR
LYQPLRLTCLWPVPGLKSSTGFVYFNATGSDVNCNGYQHNSVVDVMRYNL
NFSANSLDNLKSGVIVFKTLQYDVLFYCSNSSSGVLDTTIPFGPSSQPYY
CFINSTINTTHVSTFVGILPPTVREIVVARTGQFYINGFKYFDLGFIEAV
NFNVTTASATDFWTVAFATFVDVLVNVSATNIQNLLYCDSPFEKLQCEHL
QFGLQDGFYSANFLDDNVLPETYVALPIYYQHTDINFTATASFGGSCYVC
KPHQVNISLNGNTSVCVRTSHFSIRYIYNRVKSGSPGDSSWHIYLKSGTC
PFSFSKLNNFQKFKTICFSTVEVPGSCNFPLEATWHYTSYTIVGALYVTW
SEGNSITGVPYPVSGIREFSNLVLNNCTKYNIYDYVGTGIIRSSNQSLAG
GITYVSNSGNLLGFKNVSTGNIFIVTPCNQPDQVAVYQQSIIGAMTAVNE
SRYGLQNLLQLPNFYYVSNGGNNCTTAVMTYSNFGICADGSLIPVRPRNS
SDNGISAIITANLSIPSNWTTSVQVEYLQITSTPIVVDCATYVCNGNPRC
KNLLKQYTSACKTIEDALRLSAHLETNDVSSMLTFDSNAFSLANVTSFGD
YNLSSVLPQRNIRSSRIAGRSALEDLLFSKVVTSGLGTVDVDYKSCTKGL
SIADLACAQYYNGIMVLPGVADAERMAMYTGSLIGGMVLGGLTSAAAIPF
SLALQARLNYVALQTDVLQENQKILAASFNKAINNIVASFSSVNDAITQT
AEAIHTVTIALNKIQDVVNQQGSALNHLTSQLRHNFQAISNSIQAIYDRL
DSIQADQQVDRLITGRLAALNAFVSQVLNKYTEVRGSRRLAQQKINECVK
SQSNRYGFCGNGTHIFSIVNSAPDGLLFLHTVLLPTDYKNVKAWSGICVD
GIYGYVLRQPNLVLYSDNGVFRVTSRIMFQPRLPVLSDFVQIYNCNVTFV
NISRVELHTVIPDYVDVNKTLQEFAQNLPKYVKPNFDLTPFNLTYLNLSS
ELKQLEAKTASLFQTTVELQGLIDQINSTYVDLKLLNREN YIKWPWWVW
LIISVVFVVLLSLLVFCCLSTGCCGCCNCLTSSMRGCCDCGSTKLPYYEF EKVHVQ

An exemplary sequence of NL63-CoV S ectodomain including a double proline substitution for stabilization in the prefusion conformation is provided as SEQ ID NO: 19:

TABLE-US-00026 MKLFLILLVLPLASCFFTCNSNANLSMLQLGVPDNSSTIVTGLLPTHWFC
ANQSTSVYSANGFFYIDVGNHRSAFALHTGYYDANQYYIYVTNEIGLNAS
VTLKICKFSRNTTFDFLSNASSSFDCIVNLLFTEQLGAPLGITISGETVR
LHLYNVTRTFYVPAAYKLTKLSVKCYFNYSCVFSVVNATVTVNVTTHNGR
VVNYTVCDDCNGYTDNIFSVQQDGRIPNGFPFNNWFLLTNGSTLVDGVSR
LYQPLRLTCLWPVPGLKSSTGFVYFNATGSDVNCNGYQHNSVVDVMRYNL
NFSANSLDNLKSGVIVFKTLQYDVLFYCSNSSSGVLDTTIPFGPSSQPYY
CFINSTINTTHVSTFVGILPPTVREIVVARTGQFYINGFKYFDLGFIEAV
NFNVTTASATDFWTVAFATFVDVLVNVSATNIQNLLYCDSPFEKLQCEHL
QFGLQDGFYSANFLDDNVLPETYVALPIYYQHTDINFTATASFGGSCYVC
KPHQVNISLNGNTSVCVRTSHFSIRYIYNRVKSGSPGDSSWHIYLKSGTC
PFSFSKLNNFQKFKTICFSTVEVPGSCNFPLEATWHYTSYTIVGALYVTW
SEGNSITGVPYPVSGIREFSNLVLNNCTKYNIYDYVGTGIIRSSNQSLAG
GITYVSNSGNLLGFKNVSTGNIFIVTPCNQPDQVAVYQQSIIGAMTAVNE
SRYGLQNLLQLPNFYYVSNGGNNCTTAVMTYSNFGICADGSLIPVRPRNS
SDNGISAIITANLSIPSNWTTSVQVEYLQITSTPIVVDCATYVCNGNPRC
KNLLKQYTSACKTIEDALRLSAHLETNDVSSMLTFDSNAFSLANVTSFGD
YNLSSVLPQRNIRSSRIAGRSALEDLLFSKVVTSGLGTVDVDYKSCTKGL
SIADLACAQYYNGIMVLPGVADAERMAMYTGSLIGGMVLGGLTSAAAIPF
SLALQARLNYVALQTDVLQENQKILAASFNKAINNIVASFSSVNDAITQT
AEAIHTVTIALNKIQDVVNQQGSALNHLTSQLRHNFQAISNSIQAIYDRL
DPPQADQQVDRLITGRLAALNAFVSQVLNKYTEVRGSRRLAQQKINECVK
SQSNRYGFCGNGTHIFSIVNSAPDGLLFLHTVLLPTDYKNVKAWSGICVD
GIYGYVLRQPNLVLYSDNGVFRVTSRIMFQPRLPVLSDFVQIYNCNVTFV
NISRVELHTVIPDYVDVNKTLQEFAQNLPKYVKPNFDLTPFNLTYLNLSS
ELKQLEAKTASLFQTTVELQGLIDQINSTYVDLKLLNRFEN

A C-terminal trimerization domain can be added to the protomers of the NL63-CoV S ectodomains trimer to promote trimerization of the ectodomain.

An exemplary sequence of NL63-CoV S ectodomain including a double proline substitution for stabilization in the prefusion conformation, and a T4 fibritin trimerization domain is provided as SEQ ID NO: 36:

TABLE-US-00027 MKLFLILLVLPLASCFFTCNSNANLSMLQLGVPDNSSTIVTGLLPTHWFC
ANQSTSVYSANGFFYIDVGNHRSAFALHTGYYDANQYYIYVTNEIGLNAS
VTLKICKFSRNTTFDFLSNASSSFDCIVNLLFTEQLGAPLGITISGETVR
LHLYNVTRTFYVPAAYKLTKLSVKCYFNYSCVFSVVNATVTVNVTTHNGR
VVNYTVCDDCNGYTDNIFSVQQDGRIPNGFPFNNWFLLTNGSTLVDGVSR
LYQPLRLTCLWPVPGLKSSTGFVYFNATGSDVNCNGYQHNSVVDVMRYNL
NFSANSLDNLKSGVIVFKTLQYDVLFYCSNSSSGVLDTTIPFGPSSQPYY
CFINSTINTTHVSTFVGILPPTVREIVVARTGQFYINGFKYFDLGFIEAV
NFNVTTASATDFWTVAFATFVDVLVNVSATNIQNLLYCDSPFEKLQCEHL
QFGLQDGFYSANFLDDNVLPETYVALPIYYQHTDINFTATASFGGSCYVC
KPHQVNISLNGNTSVCVRTSHFSIRYIYNRVKSGSPGDSSWHIYLKSGTC
PFSFSKLNNFQKFKTICFSTVEVPGSCNFPLEATWHYTSYTIVGALYVTW
SEGNSITGVPYPVSGIREFSNLVLNNCTKYNIYDYVGTGIIRSSNQSLAG
GITYVSNSGNLLGFKNVSTGNIFIVTPCNQPDQVAVYQQSIIGAMTAVNE
SRYGLQNLLQLPNFYYVSNGGNNCTTAVMTYSNFGICADGSLIPVRPRNS
SDNGISAIITANLSIPSNWTTSVQVEYLQITSTPIVVDCATYVCNGNPRC
KNLLKQYTSACKTIEDALRLSAHLETNDVSSMLTFDSNAFSLANVTSFGD
YNLSSVLPQRNIRSSRIAGRSALEDLLFSKVVTSGLGTVDVDYKSCTKGL
SIADLACAQYYNGIMVLPGVADAERMAMYTGSLIGGMVLGGLTSAAAIPF
SLALQARLNYVALQTDVLQENQKILAASFNKAINNIVASFSSVNDAITQT
AEAIHTVTIALNKIQDVVNQQGSALNHLTSQLRHNFQAISNSIQAIYDRL
DPPQADQQVDRLITGRLAALNAFVSQVLNKYTEVRGSRRLAQQKINECVK
SQSNRYGFCGNGTHIFSIVNSAPDGLLFLHTVLLPTDYKNVKAWSGICVD
GIYGYVLRQPNLVLYSDNGVFRVTSRIMFQPRLPVLSDFVQIYNCNVTFV
NISRVELHTVIPDYVDVNKTLQEFAQNLPKYVKPNFDLTPFNLTYLNLSS
ELKQLEAKTASLFQTTVELQGLIDQINSTYVDLKLLNRFENGGYIPEAPR DGQAYVRKDGEWVLLSTF

In some embodiments, the recombinant NL63-CoV S ectodomain trimer comprises protomers comprising the ectodomain sequence of SEQ ID NO: 19. In some embodiments, the recombinant NL63-CoV S ectodomain trimer comprises protomers comprising residues 16-1291 of SEQ ID NO: 19 or residues 16-1318 of SEQ ID NO: 36. In some embodiments, the recombinant NL63-CoV S ectodomain trimer comprises protomers comprising an ectodomain sequence at least 90% identical to the ectodomain sequence of SEQ ID NO: 19, wherein the NL63-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers. In some embodiments, the recombinant NL63-CoV S ectodomain trimer comprises protomers comprising an amino acid sequence at least 90% identical to residues 16-1291 of SEQ ID NO: 19 or residues 16-1318 of SEQ ID NO: 36, wherein the NL63-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers.

I. 229E-CoV

In some embodiments, the immunogen comprises a recombinant 229E-CoV S ectodomain trimer comprising protomers comprising one or more (such as two, for example two consecutive) proline substitutions at or near the boundary between a HR1 domain and a central helix domain that stabilize the S ectodomain trimer in the prefusion conformation. In some such embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the S ectodomain in the prefusion conformation are located between a position 15 amino acids N-terminal of a C-terminal residue of the HR1 and a position 5 amino acids C-terminal of a N-terminal residue of the central helix.

In some embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the 229E-CoV S ectodomain trimer in the prefusion conformation are located between residues 852 to 872 (such as between residues 862 to 872 or between residues 867 to 872) of the S ectodomain protomers in the trimer. In some embodiments, the 229E-CoV S ectodomain trimer is stabilized in the prefusion conformation by I869P and I870P substitutions ("2P") in the S ectodomain protomers in the trimer. The amino acid numbering for 229E-CoV S proteins is with reference to the 229E-CoV S sequence provided as SEQ ID NO: 20.

In some embodiments, the recombinant 229E-CoV S ectodomain trimer stabilized in the prefusion conformation comprises single-chain S ectodomain protomers comprising mutations to the S1/S2 and/or S2' protease cleavage sites to prevent protease cleavage at these sites.

In some embodiments, the protomers of the recombinant 229E-CoV S ectodomain trimer stabilized in the prefusion conformation by the one or more proline substitutions (such as I869P and 1870P substitutions) comprises additional modifications for stabilization in the prefusion conformation.

With reference to the 229E-CoV S protein sequence provided as SEQ ID NO: 20, the ectodomain of the 229E-CoV S protein includes about residues 17-1108. Residues 1-16 are the signal peptide, which is removed during cellular processing. The S1/S2 cleavage site is located at about position 565/566. The S2' cleavage site is located at about position 687/688. The HR1 is located at about residues 784-866. The central helix is located at about residues 871-912. The HR2 is located at about 1050-1094. The C-terminal end of the S2 ectodomain is located at about residue 1108. In some embodiments, the protomers of the prefusion-stabilized 229E-CoV S ectodomain trimer can have a C-terminal residue (which can be linked to a trimerization domain, or a transmembrane domain, for example) of the C-terminal residue of the HR2 (e.g., position 1099), or the ectodomain (e.g., position 1108), or from one of positions 1099-1108. The position numbering of the S protein may vary between 229E-CoV stains, but the sequences can be aligned to determine relevant structural domains and cleavage sites. It will be appreciated that a few residues (such as up to 10) on the N and C-terminal ends of the ectodomain can be removed or modified in the disclosed immunogens without decreasing the utility of the S ectodomain trimer as an immunogen.

Exemplary 229E-CoV S protein sequences are provided below. The prefusion stabilizing substitutions disclosed herein (and other modifications, such as substitutions to generate a single chain) can be incorporated into 229E-CoV S protein sequences.

An exemplary sequence of 229E-CoV S protein (including the ectodomain and TM and CT domains) is provided as SEQ ID NO: 20 (GenBank GI: 1060650120, incorporated by reference herein):

TABLE-US-00028 MFVLLVAYALLHIAGCQTTNGTNTSHSVCNGCVGHSENVFAVESGGYIPS
NFAFNNWFLLTNTSSVVDGVVRSFQPLLLNCLWSVSGSQFTTGFVYFNGT
GRGACKGFYSNASSDVIRYNINFEENLRRGTILFKTSYGAVVFYCTNNTL
VSGDAHIPSGTVLGNFYCFVNTTIGNETTSAFVGALPKTVREFVISRTGH
FYINGYRYFSLGDVEAVNFNVTNAATTVCTVALASYADVLVNVSQTAIAN
IIYCNSVINRLRCDQLSFDVPDGFYSTSPIQPVELPVSIVSLPVYHKHTF
IVLYVNFEHRRGPGKCYNCRPAVINITLANFNETKGPLCVDTSHFTTQFV
DNVKLARWSASINTGNCPFSFGKVNNFVKFGSVCFSLKDIPGGCAMPIMA
NLVNSKSHNIGSLYVSWSDGDVITGVPKPVEGVSSFMNVTLNKCTKYNIY
DVSGVGVIRISNDTFLNGITYTSTSGNLLGFKDVTNGTIYSITPCNPPDQ
LVVYQQAVVGAMLSENFTSYGFSNVVEMPKFFYASNGTYNCTDAVLTYSS
FGVCADGSIIAVQPRNVSYDSVSAIVTANLSIPFNWTTSVQVEYLQITST
PIVVDCSTYVCNGNVRCVELLKQYTSACKTIEDALRNSAMLESADVSEML
TFDKKAFTLANVSSFGDYNLSSVIPSLPRSGSRVAGRSAIEDILFSKLVT
SGLGTVDADYKKCTKGLSIADLACAQYYNGIMVLPGVADAERMAMYTGSL
IGGIALGGLTSAASIPFSLAIQSRLNYVALQTDVLQENQRILAASFNKAM
TNIVDAFTGVNDAITQTSQALQTVATALNKIQDVVNQQGNSLNHLTSQLR
QNFQAISSSIQAIYDRLDIIQADQQVDRLITGRLAALNVFVSHTLTKYTE
VRASRQLAQQKVNECVKSQSKRYGFCGNGTHIFSLVNAAPEGLVFLHTVL
LPTQYKDVEAWSGLCVDGINGYVLRQPNLALYKEGNYYRITSRIMFEPRI
PTIADFVQIENCNVTFVNISRSELQTIVPEYIDVNKTLQELSYKLPNYTV
PDLVVEQYNQTILNLTSEISTLENKSAELNYTVQKLQTLIDNINSTLVDL
KWLNRVETYIKWPWWVWLCISVVLIFVVSMLLLCCCSTGCCGFFSCFASS IRGCCESTKLPYYDVEKIHIQ

An exemplary sequence of 229E-CoV S ectodomain including a double proline substitution for stabilization in the prefusion conformation is provided as SEQ ID NO: 21:

TABLE-US-00029 MFVLLVAYALLHIAGCQTTNGTNTSHSVCNGCVGHSENVFAVESGGYIPS
NFAFNNWFLLTNTSSVVDGVVRSFQPLLLNCLWSVSGSQFTTGFVYFNGT
GRGACKGFYSNASSDVIRYNINFEENLRRGTILFKTSYGAVVFYCTNNTL
VSGDAHIPSGTVLGNFYCFVNTTIGNETTSAFVGALPKTVREFVISRTGH
FYINGYRYFSLGDVEAVNFNVTNAATTVCTVALASYADVLVNVSQTAIAN
IIYCNSVINRLRCDQLSFDVPDGFYSTSPIQPVELPVSIVSLPVYHKHTF
IVLYVNFEHRRGPGKCYNCRPAVINITLANFNETKGPLCVDTSHFTTQFV
DNVKLARWSASINTGNCPFSFGKVNNFVKFGSVCFSLKDIPGGCAMPIMA
NLVNSKSHNIGSLYVSWSDGDVITGVPKPVEGVSSFMNVTLNKCTKYNIY
DVSGVGVIRISNDTFLNGITYTSTSGNLLGFKDVTNGTIYSITPCNPPDQ
LVVYQQAVVGAMLSENFTSYGFSNVVEMPKFFYASNGTYNCTDAVLTYSS
FGVCADGSIIAVQPRNVSYDSVSAIVTANLSIPFNWTTSVQVEYLQITST

PIVVDCSTYVCNGNVRCVELLKQYTSACKTIEDALRNSAMLESADVSEML
TFDKKAFTLANVSSFGDYNLSSVIPSLPRSGSRVAGRSAIEDILFSKLVT
SGLGTVDADYKKCTKGLSIADLACAQYYNGIMVLPGVADAERMAMYTGSL
IGGIALGGLTSAASIPFSLAIQSRLNYVALQTDVLQENQRILAASFNKAM
TNIVDAFTGVNDAITQTSQALQTVATALNKIQDVVNQQGNSLNHLTSQLR
QNFQAISSSIQAIYDRLDPPQADQQVDRLITGRLAALNVFVSHTLTKYTE
VRASRQLAQQKVNECVKSQSKRYGFCGNGTHIFSLVNAAPEGLVFLHTVL
LPTQYKDVEAWSGLCVDGINGYVLRQPNLALYKEGNYYRITSRIMFEPRI
PTIADFVQIENCNVTFVNISRSELQTIVPEYIDVNKTLQELSYKLPNYTV
PDLVVEQYNQTILNLTSEISTLENKSAELNYTVQKLQTLIDNINSTLVDL KWLNRVET

A C-terminal trimerization domain can be added to the protomers of the 229E-CoV S ectodomains trimer to promote trimerization of the ectodomain.

An exemplary sequence of 229E-CoV S ectodomain including a double proline substitution for stabilization in the prefusion conformation, and a T4 fibritin trimerization domain is provided as SEQ ID NO: 37:

TABLE-US-00030 MFVLLVAYALLHIAGCQTTNGTNTSHSVCNGCVGHSENVFAVESGGYIPS
NFAFNNWFLLTNTSSVVDGVVRSFQPLLLNCLWSVSGSQFTTGFVYFNGT
GRGACKGFYSNASSDVIRYNINFEENLRRGTILFKTSYGAVVFYCTNNTL
VSGDAHIPSGTVLGNFYCFVNTTIGNETTSAFVGALPKTVREFVISRTGH
FYINGYRYFSLGDVEAVNFNVTNAATTVCTVALASYADVLVNVSQTAIAN
IIYCNSVINRLRCDQLSFDVPDGFYSTSPIQPVELPVSIVSLPVYHKHTF
IVLYVNFEHRRGPGKCYNCRPAVINITLANFNETKGPLCVDTSHFTTQFV
DNVKLARWSASINTGNCPFSFGKVNNFVKFGSVCFSLKDIPGGCAMPIMA
NLVNSKSHNIGSLYVSWSDGDVITGVPKPVEGVSSFMNVTLNKCTKYNIY
DVSGVGVIRISNDTFLNGITYTSTSGNLLGFKDVTNGTIYSITPCNPPDQ
LVVYQQAVVGAMLSENFTSYGFSNVVEMPKFFYASNGTYNCTDAVLTYSS
FGVCADGSIIAVQPRNVSYDSVSAIVTANLSIPFNWTTSVQVEYLQITST
PIVVDCSTYVCNGNVRCVELLKQYTSACKTIEDALRNSAMLESADVSEML
TFDKKAFTLANVSSFGDYNLSSVIPSLPRSGSRVAGRSAIEDILFSKLVT
SGLGTVDADYKKCTKGLSIADLACAQYYNGIMVLPGVADAERMAMYTGSL
IGGIALGGLTSAASIPFSLAIQSRLNYVALQTDVLQENQRILAASFNKAM
TNIVDAFTGVNDAITQTSQALQTVATALNKIQDVVNQQGNSLNHLTSQLR
QNFQAISSSIQAIYDRLDPPQADQQVDRLITGRLAALNVFVSHTLTKYTE
VRASRQLAQQKVNECVKSQSKRYGFCGNGTHIFSLVNAAPEGLVFLHTVL
LPTQYKDVEAWSGLCVDGINGYVLRQPNLALYKEGNYYRITSRIMFEPRI
PTIADFVQIENCNVTFVNISRSELQTIVPEYIDVNKTLQELSYKLPNYTV
PDLVVEQYNQTILNLTSEISTLENKSAELNYTVQKLQTLIDNINSTLVDL
KWLNRVETGGYIPEAPRDGQAYVRKDGEWVLLSTF

In some embodiments, the recombinant 229E-CoV S ectodomain trimer comprises protomers comprising the ectodomain sequence of SEQ ID NO: 21. In some embodiments, the recombinant 229E-CoV S ectodomain trimer comprises protomers comprising residues 17-1108 of SEQ ID NO: 21 or residues 17-1135 of SEQ ID NO: 37. In some embodiments, the recombinant 229E-CoV S ectodomain trimer comprises protomers comprising an ectodomain sequence at least 90% identical to the ectodomain sequence of SEQ ID NO: 21, wherein the 229E-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers. In some embodiments, the recombinant 229E-CoV S ectodomain trimer comprises protomers comprising an amino acid sequence at least 90% identical to residues 17-1108 of SEQ ID NO: 21 or residues 17-1135 of SEQ ID NO: 37, wherein the 229E-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers.

I. PEDV-CoV

In some embodiments, the immunogen comprises a recombinant PEDV-CoV S ectodomain trimer comprising protomers comprising one or more (such as two, for example two consecutive) proline substitutions at or near the boundary between a HR1 domain and a central helix domain that stabilize the S ectodomain trimer in the prefusion conformation. In some such embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the S ectodomain in the prefusion conformation are located between a position 15 amino acids N-terminal of a C-terminal residue of the HR1 and a position 5 amino acids C-terminal of a N-terminal residue of the central helix.

In some embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the PEDV-CoV

S ectodomain trimer in the prefusion conformation are located between residues 1059 to 1079 (such as between residues 1069 to 1079 or between residues 1073 to 1079) of the S ectodomain protomers in the trimer. In some embodiments, the PEDV-CoV S ectodomain trimer is stabilized in the prefusion conformation by I1076P and L1077P substitutions ("2P") in the S ectodomain protomers in the trimer. The amino acid numbering for PEDV-CoV S proteins is with reference to the PEDV-CoV S sequence provided as SEQ ID NO: 38.

In some embodiments, the recombinant PEDV-CoV S ectodomain trimer stabilized in the prefusion conformation comprises single-chain S ectodomain protomers comprising mutations to the S1/S2 and/or S2' protease cleavage sites to prevent protease cleavage at these sites.

In some embodiments, the protomers of the recombinant PEDV-CoV S ectodomain trimer stabilized in the prefusion conformation by the one or more proline substitutions (such as I1076P and L1077P substitutions) comprises additional modifications for stabilization in the prefusion conformation.

With reference to the PEDV-CoV S protein sequence provided as SEQ ID NO: 38, the ectodomain of the PEDV-CoV S protein includes about residues 21-1322. Residues 1-20 are the signal peptide, which is removed during cellular processing. The S1/S2 cleavage site is located at about position 736/737. The S2' cleavage site is located at about position 743/744. The HR1 is located at about residues 991-1073. The central helix is located at about residues 1078-1119. The HR2 is located at about 1277-1308. The C-terminal end of the S2 ectodomain is located at about residue 1322. In some embodiments, the protomers of the prefusion-stabilized PEDV-CoV S ectodomain trimer can have a C-terminal residue (which can be linked to a trimerization domain, or a transmembrane domain, for example) of the C-terminal residue of the HR2 (e.g., position 1308), or the ectodomain (e.g., position 1322), or from one of positions 1308-1322. The position numbering of the S protein may vary between PEDV-CoV stains, but the sequences can be aligned to determine relevant structural domains and cleavage sites. It will be appreciated that a few residues (such as up to 10) on the N and C-terminal ends of the ectodomain can be removed or modified in the disclosed immunogens without decreasing the utility of the S ectodomain trimer as an immunogen.

Exemplary PEDV-CoV S protein sequences are provided below. The prefusion stabilizing substitutions disclosed herein (and other modifications, such as substitutions to generate a single chain) can be incorporated into PEDV-CoV S protein sequences.

An exemplary sequence of PEDV-CoV S protein (including the ectodomain and TM and CT domains) is provided as SEQ ID NO: 38 (GenBank GI: AHZ94887.1, incorporated by reference herein):

TABLE-US-00031 MKSLTYFWLFLPVLSTLSLPQDVTRCSANTNFRRFFSKFNVQAPAVVVLG
GYLPIGENQGVNSTWYCAGQHPTASGVHGIFVSHIRGGHGFEIGISQEPF
DPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLF
NKAIPAHMSEHSVVGITWDNDRVTVFSDKIYYFYFKNDWSRVATKCYNSG
GCAMQYVYEPTYYMLNVTSAGEDGISYQPCTANCIGYAANVFATEPNGHI
PEGFSFNNWFLLSNDSTLVHGKVVSNQPLLVNCLLAIPKIYGLGQFFSFN
QTIDGVCNGAAVQRAPEALRFNINDISVILAEGSIVLHTALGTNFSFVCS
NSSNPHLATFAIPLGATQVPYYCFFKVDTYNSTVYKFLAVLPPTVREIVI
TKYGDVYVNGFGYLHLGLLDAVTINFTGHGTDDDVSGFWTIASTNFVDAL
IEVQGTAIQRILYCDDPVSQLKCSQVAFDLDDGFYTISSRNLLSHEQPIS
FVTLPSFNDHSFVNITVSASFGGHSGANLIASDTTINGFSSFCVDTRQFT
ISLFYNVTNSYGYVSKSQDSNCPFTLQSVNDYLSFSKFCVSTSLLASACT
IDLFGYPEFGSGVKFTSLYFQFTKGELITGTPKPLEGVTDVSFMTLDVCT
KYTIYGFKGEGIITLTNSSFLAGVYYTSDSGQLLAFKNVTSGAVYSVTPC
SFSEQAAYVDDDIVGVISSLSSSTFNSTRELPGFFYHSNDGSNCTEPVLV
YSNIGVCKSGSIGYVPSQSGQVKIAPTVTGNISIPTNFSMSIRTEYLQLY
NTPVSVDCATYVCNGNSRCKQLLTQYTAACKTIESALQLSARLESVEVNS
MLTISDEALQLATISSFNGDGYNFTNVLGVSVYDPASRRVVQKRSFIEDL
LFNKVVTNGLGTVDEDYKRCSNGRSVADLVCAQYYSGVMVLPGVVDAEKL
HMYSASLIGGMVLGGFTSAAALPFSYAVQARLNYLALQTDVLQRNQQLLA
ESFNSAIGNITSAFESVKEAISQTSKGLNTVAHALTKVQEVVNSQGAALT
QLTVQLQHNFQAISSSIDDIYSRLDILSADAQVDRLITGRLSALNAFVAQ
TLTKYTEVQASRKLAQQKVNECVKSQSQRYGFCGGDGEHIFSLVQAAPQG
LLFLHTVLVPSDFVDVIAIAGLCVNDEIALTLREPGLVLFTHELQNHTAT
EYFVSSRRMFEPRKPTVSDFVQIESCVVTYVNLTRDQLPDVIPDYIDVNK
TLYEILASLPNRTGPSLPLDVFNATYLNLTGEIADLEQRSESLRNTTEEL
QSLIYNINNTLVDLEWLNRVETYIKWPWWVWLIIFIVLIFVVSLLVFCCI
STGCCGCCGCCCACFSGCCRGPRLQPYEVFEKVHVQ

An exemplary sequence of PEDV-CoV S ectodomain including a double proline substitution for stabilization in the prefusion

conformation is provided as SEQ ID NO: 39:

TABLE-US-00032 MKSLTYFWLFLPVLSTLSLPQDVTRCSANTNFRRFFSKFNVQAPAVVVLG
GYLPIGENQGVNSTWYCAGQHPTASGVHGIFVSHIRGGHGFEIGISQEPF
DPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLF
NKAIPAHMSEHSVVGITWDNDRVTVFSDKIYYFYFKNDWSRVATKCYNSG
GCAMQYVYEPTYYMLNVTSAGEDGISYQPCTANCIGYAANVFATEPNGHI
PEGFSFNNWFLLSNDSTLVHGKVVSNQPLLVNCLLAIPKIYGLGQFFSFN
QTIDGVCNGAAVQRAPEALRFNINDISVILAEGSIVLHTALGTNFSFVCS
NSSNPHLATFAIPLGATQVPYYCFFKVDTYNSTVYKFLAVLPPTVREIVI
TKYGDVYVNGFGYLHLGLLDAVTINFTGHGTDDDVSGFWTIASTNFVDAL
IEVQGTAIQRILYCDDPVSQLKCSQVAFDLDDGFYTISSRNLLSHEQPIS
FVTLPSFNDHSFVNITVSASFGGHSGANLIASDTTINGFSSFCVDTRQFT
ISLFYNVTNSYGYVSKSQDSNCPFTLQSVNDYLSFSKFCVSTSLLASACT
IDLFGYPEFGSGVKFTSLYFQFTKGELITGTPKPLEGVTDVSFMTLDVCT
KYTIYGFKGEGIITLTNSSFLAGVYYTSDSGQLLAFKNVTSGAVYSVTPC
SFSEQAAYVDDDIVGVISSLSSSTFNSTRELPGFFYHSNDGSNCTEPVLV
YSNIGVCKSGSIGYVPSQSGQVKIAPTVTGNISIPTNFSMSIRTEYLQLY
NTPVSVDCATYVCNGNSRCKQLLTQYTAACKTIESALQLSARLESVEVNS
MLTISDEALQLATISSFNGDGYNFTNVLGVSVYDPASRRVVQKRSFIEDL
LFNKVVTNGLGTVDEDYKRCSNGRSVADLVCAQYYSGVMVLPGVVDAEKL
HMYSASLIGGMVLGGFTSAAALPFSYAVQARLNYLALQTDVLQRNQQLLA
ESFNSAIGNITSAFESVKEAISQTSKGLNTVAHALTKVQEVVNSQGAALT
QLTVQLQHNFQAISSSIDDIYSRLDPPSADAQVDRLITGRLSALNAFVAQ
TLTKYTEVQASRKLAQQKVNECVKSQSQRYGFCGGDGEHIFSLVQAAPQG
LLFLHTVLVPSDFVDVIAIAGLCVNDEIALTLREPGLVLFTHELQNHTAT
EYFVSSRRMFEPRKPTVSDFVQIESCVVTYVNLTRDQLPDVIPDYIDVNK
TLYEILASLPNRTGPSLPLDVFNATYLNLTGEIADLEQRSESLRNTTEEL QSLIYNINNTLVDLEWLNRVET

A C-terminal trimerization domain can be added to the protomers of the PEDV-CoV S ectodomains trimer to promote trimerization of the ectodomain.

An exemplary sequence of PEDV-CoV S ectodomain including a double proline substitution for stabilization in the prefusion conformation, and a T4 fibritin trimerization domain is provided as SEQ ID NO: 40:

TABLE-US-00033 MKSLTYFWLFLPVLSTLSLPQDVTRCSANTNFRRFFSKFNVQAPAVVVLG
GYLPIGENQGVNSTWYCAGQHPTASGVHGIFVSHIRGGHGFEIGISQEPF
DPSGYQLYLHKATNGNTNATARLRICQFPSIKTLGPTANNDVTTGRNCLF
NKAIPAHMSEHSVVGITWDNDRVTVFSDKIYYFYFKNDWSRVATKCYNSG
GCAMQYVYEPTYYMLNVTSAGEDGISYQPCTANCIGYAANVFATEPNGHI
PEGFSFNNWFLLSNDSTLVHGKVVSNQPLLVNCLLAIPKIYGLGQFFSFN
QTIDGVCNGAAVQRAPEALRFNINDISVILAEGSIVLHTALGTNFSFVCS
NSSNPHLATFAIPLGATQVPYYCFFKVDTYNSTVYKFLAVLPPTVREIVI
TKYGDVYVNGFGYLHLGLLDAVTINFTGHGTDDDVSGFWTIASTNFVDAL
IEVQGTAIQRILYCDDPVSQLKCSQVAFDLDDGFYTISSRNLLSHEQPIS
FVTLPSFNDHSFVNITVSASFGGHSGANLIASDTTINGFSSFCVDTRQFT
ISLFYNVTNSYGYVSKSQDSNCPFTLQSVNDYLSFSKFCVSTSLLASACT
IDLFGYPEFGSGVKFTSLYFQFTKGELITGTPKPLEGVTDVSFMTLDVCT
KYTIYGFKGEGIITLTNSSFLAGVYYTSDSGQLLAFKNVTSGAVYSVTPC
SFSEQAAYVDDDIVGVISSLSSSTFNSTRELPGFFYHSNDGSNCTEPVLV
YSNIGVCKSGSIGYVPSQSGQVKIAPTVTGNISIPTNFSMSIRTEYLQLY
NTPVSVDCATYVCNGNSRCKQLLTQYTAACKTIESALQLSARLESVEVNS
MLTISDEALQLATISSFNGDGYNFTNVLGVSVYDPASRRVVQKRSFIEDL
LFNKVVTNGLGTVDEDYKRCSNGRSVADLVCAQYYSGVMVLPGVVDAEKL
HMYSASLIGGMVLGGFTSAAALPFSYAVQARLNYLALQTDVLQRNQQLLA
ESFNSAIGNITSAFESVKEAISQTSKGLNTVAHALTKVQEVVNSQGAALT
QLTVQLQHNFQAISSSIDDIYSRLDPPSADAQVDRLITGRLSALNAFVAQ
TLTKYTEVQASRKLAQQKVNECVKSQSQRYGFCGGDGEHIFSLVQAAPQG
LLFLHTVLVPSDFVDVIAIAGLCVNDEIALTLREPGLVLFTHELQNHTAT
EYFVSSRRMFEPRKPTVSDFVQIESCVVTYVNLTRDQLPDVIPDYIDVNK
TLYEILASLPNRTGPSLPLDVFNATYLNLTGEIADLEQRSESLRNTTEEL
QSLIYNINNTLVDLEWLNRVETGGYIPEAPRDGQAYVRKDGEWVLLSTF

In some embodiments, the recombinant PEDV-CoV S ectodomain trimer comprises protomers comprising the ectodomain sequence of SEQ ID NO: 39. In some embodiments, the recombinant PEDV-CoV S ectodomain trimer comprises protomers comprising residues 21-1322 of SEQ ID NO: 39 or residues 21-1349 of SEQ ID NO: 40. In some embodiments, the recombinant PEDV-CoV S ectodomain trimer comprises protomers comprising an ectodomain sequence at least 90% identical to the ectodomain sequence of SEQ ID NO: 39, wherein the PEDV-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers. In some embodiments, the recombinant PEDV-CoV S ectodomain trimer comprises protomers comprising an amino acid sequence at least 90% identical to residues 21-1322 of SEQ ID NO: 39 or residues 21-1349 of SEQ ID NO: 40, wherein the PEDV-CoV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers.

I. SDCV

In some embodiments, the immunogen comprises a recombinant swine delta coronavirus (SDCV) S ectodomain trimer comprising protomers comprising one or more (such as two, for example two consecutive) proline substitutions at or near the boundary between a HR1 domain and a central helix domain that stabilize the S ectodomain trimer in the prefusion conformation. In some such embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the S ectodomain in the prefusion conformation are located between a position 15 amino acids N-terminal of a C-terminal residue of the HR1 and a position 5 amino acids C-terminal of a N-terminal residue of the central helix.

In some embodiments, the one or more (such as two, for example two consecutive) proline substitutions that stabilize the SDCV S ectodomain trimer in the prefusion conformation are located between residues 838 to 858 (such as between residues 848 to 858 or between residues 854 to 858) of the S ectodomain protomers in the trimer. In some embodiments, the SDCV S ectodomain trimer is stabilized in the prefusion conformation by E855P and V856P substitutions ("2P") in the S ectodomain protomers in the trimer. The amino acid numbering for SDCV S proteins is with reference to the SDCV S sequence provided as SEQ ID NO: 41.

In some embodiments, the recombinant SDCV S ectodomain trimer stabilized in the prefusion conformation comprises single-chain S ectodomain protomers comprising mutations to the S1/S2 and/or S2' protease cleavage sites to prevent protease cleavage at these sites.

In some embodiments, the protomers of the recombinant SDCV S ectodomain trimer stabilized in the prefusion conformation by the one or more proline substitutions (such as E855P and V856P substitutions) comprises additional modifications for stabilization in the prefusion conformation.

With reference to the SDCV S protein sequence provided as SEQ ID NO: 41, the ectodomain of the SDCV S protein includes about residues 20-1093. Residues 1-19 are the signal peptide, which is removed during cellular processing. The HR1 is located at about residues 770-854. The central helix is located at about residues 857-898. The HR2 is located at about 1034-1079. The C-terminal end of the S2 ectodomain is located at about residue 1093. In some embodiments, the protomers of the prefusion-stabilized SDCV S ectodomain trimer can have a C-terminal residue (which can be linked to a trimerization domain, or a transmembrane domain, for example) of the C-terminal residue of the HR2 (e.g., position 1079), or the ectodomain (e.g., position 1093), or from one of positions 1079-1093. The position numbering of the S protein may vary between SDCV stains, but the sequences can be aligned to determine relevant structural domains and cleavage sites. It will be appreciated that a few residues (such as up to 10) on the N and C-terminal ends of the ectodomain can be removed or modified in the disclosed immunogens without decreasing the utility of the S ectodomain trimer as an immunogen.

Exemplary SDCV S protein sequences are provided below. The prefusion stabilizing substitutions disclosed herein (and other modifications, such as substitutions to generate a single chain) can be incorporated into SDCV S protein sequences.

An exemplary sequence of SDCV S protein (including the ectodomain and TM and CT domains) is provided as SEQ ID NO: 41 (GenBank GI: AMN91621.1, incorporated by reference herein):

TABLE-US-00034 MQRALLIMTLLCLARAKFADDLLDLLTFPGAHRFLHKPTRNDSILYSRAN
NNFDVGVLPGYPTKNVNLFSPLTNSTLPINGLHRSYQPLMLNCLTKITNQ TLSMYLQPSEIQTYSCGGAMVKYQTHDAVRIILDLIATDRISVEVVGQAG
ENYVFVCSDQFNYTTALHNSTFFSLNSQLYCFTNNTYLGILPPDLTDFTV YRTGQFYANGYLLGTLPITVNYVRLYRGQLSANSAHFALANLTDTLITLT
NTTISQITYCDKSVVDSIACQRSSHQVEDGFYSDPKSAVRARQRTIVTLP KLPELEVVQLNISAHMDFGEARLDSVTINGNTSYCVTKPYFRLETNFLCR
GCTMNLRTDTCSFDLSAVNNGMSFSQFCLSTESGACEMKIIVTYVWNYLL RQRLYVTAVEGQTHTGTTSVHATDTSSVITDVCTDYTIYGVSGTGIIKPS
DLLLHNGIAFTSPTGELYAFKNITTGKTLQVLPCETPSQLIVINNTVVGA ITSSNSTENNRFTTTIVTPTFFYSTNATTLNCTKPVLSYGPISVCSDGAI
AGTSTLQNTRPSIVSLYDGEIEIPSAFSLSVQTEYLQVQAEQVIVDCPQY VCNGNSRCLQLLAQYTSACSNIEVALHSSAQLDSREIISMFKTSTQSLQL
ANITNFKGDYNFSSILTSRVGGRSAIEDLLFNKVVTSGLGTVDQDYKSCS RNMAIADLVCSQYYNGIMVLPGVVDAEKMAMYTGSLTGAMVFGGLTAAAA
IPFATAVQARLNYVALQTNVLQENQKILAESFNQAVGNISLALSSVNDAI QQTSEALNTVAIAIKKIQTVVNQQGEALSHLTAQLSNNFQAISTSIQDIY
NRLEEVEANQQVDRLINGRLAALNAYVTQLLNQMSQIRQSRLLAQQKINE CVKSQSPRYGFCGNGTHIFSLTQTAPNGIFFMHAVLVPNKFTRVNASAGI
CVDNTRGYSLQPQLILYQFNNSWRVTPRNMYEPRLPRQADFIQLTDCSVT FYNTTAANLPNIIPDVIDVNQTVSDIIDNLPTATPPQWDVGIYNNTILNL
TVEINDLQERSKNLSQIADRLQNYIDNLNNTLVDLEWLNRVETYLKWPWY IWLAIALALIAFVTILITIFLCTGCCGGCFGCCGGCFGLFSKKKRYTDDQ
PTPSFKFKEW

An exemplary sequence of SDCV S ectodomain including a double proline substitution for stabilization in the prefusion conformation is provided as SEQ ID NO: 42:

TABLE-US-00035 MQRALLIMTLLCLARAKFADDLLDLLTFPGAHRFLHKPTRNDSILYSRAN
NNFDVGVLPGYPTKNVNLFSPLTNSTLPINGLHRSYQPLMLNCLTKITNQ TLSMYLQPSEIQTYSCGGAMVKYQTHDAVRIILDLIATDRISVEVVGQAG
ENYVFVCSDQFNYTTALHNSTFFSLNSQLYCFTNNTYLGILPPDLTDFTV YRTGQFYANGYLLGTLPITVNYVRLYRGQLSANSAHFALANLTDTLITLT
NTTISQITYCDKSVVDSIACQRSSHQVEDGFYSDPKSAVRARQRTIVTLP KLPELEVVQLNISAHMDFGEARLDSVTINGNTSYCVTKPYFRLETNFLCR
GCTMNLRTDTCSFDLSAVNNGMSFSQFCLSTESGACEMKIIVTYVWNYLL RQRLYVTAVEGQTHTGTTSVHATDTSSVITDVCTDYTIYGVSGTGIIKPS

DLLLHNGIAFTSPTGELYAFKNITTGKTLQVLPCETPSQLIVINNTVVGA ITSSNSTENNRFTTTIVTPTFFYSTNATTLNCTKPVLSYGPISVCSDGAI
AGTSTLQNTRPSIVSLYDGEIEIPSAFSLSVQTEYLQVQAEQVIVDCPQY VCNGNSRCLQLLAQYTSACSNIEVALHSSAQLDSREIISMFKTSTQSLQL
ANITNFKGDYNFSSILTSRVGGRSAIEDLLFNKVVTSGLGTVDQDYKSCS RNMAIADLVCSQYYNGIMVLPGVVDAEKMAMYTGSLTGAMVFGGLTAAAA
IPFATAVQARLNYVALQTNVLQENQKILAESFNQAVGNISLALSSVNDAI QQTSEALNTVAIAIKKIQTVVNQQGEALSHLTAQLSNNFQAISTSIQDIY
NRLEPPEANQQVDRLINGRLAALNAYVTQLLNQMSQIRQSRLLAQQKINE CVKSQSPRYGFCGNGTHIFSLTQTAPNGIFFMHAVLVPNKFTRVNASAGI
CVDNTRGYSLQPQLILYQFNNSWRVTPRNMYEPRLPRQADFIQLTDCSVT FYNTTAANLPNIIPDVIDVNQTVSDIIDNLPTATPPQWDVGIYNNTILNL
TVEINDLQERSKNLSQIADRLQNYIDNLNNTLVDLEWLNRVET

A C-terminal trimerization domain can be added to the protomers of the SDCV S ectodomains trimer to promote trimerization of the ectodomain.

An exemplary sequence of SDCV S ectodomain including a double proline substitution for stabilization in the prefusion conformation, and a T4 fibritin trimerization domain is provided as SEQ ID NO: 43:

TABLE-US-00036 MQRALLIMTLLCLARAKFADDLLDLLTFPGAHRFLHKPTRNDSILYSRAN
NNFDVGVLPGYPTKNVNLFSPLTNSTLPINGLHRSYQPLMLNCLTKITNQ TLSMYLQPSEIQTYSCGGAMVKYQTHDAVRIILDLIATDRISVEVVGQAG
ENYVFVCSDQFNYTTALHNSTFFSLNSQLYCFTNNTYLGILPPDLTDFTV YRTGQFYANGYLLGTLPITVNYVRLLYRGQLSANSAHFALANLTDTLITLT
NTTISQITYCDKSVVDSIACQRSSHQVEDGFYSDPKSAVRARQRTIVTLP KLPELEVVQLNISAIHMDFGEARLDSVTINGNTSYCVTKPYFRLETNFLCR
GCTMNLRTDTCSFDLSAVNNGMSFSQFCLSTESGACEMKHVTVVWNYLL RQRLYVTAVEGQTHTGTTSVHATDTSSVITDVCTDYTYGVSGTGIIKPS
DLLLHNGIAFTSPTGELYAFKNITTGKTLQVLPCETPSQLIVINNTVVGA ITSSNSTENNRFTTTIVTPTFFYSTNATTLNCTKPVLSYGPISVCSDGAI
AGTSTLQNTRPSIVSLYDGEIEIPSAFSLSVQTEYLQVQAEQVIVDCPQY VCNGNSRCLQLLAQYTSACSNIEVALHSSAQLDSREIISMFKTSTQSLQL
ANITNFKGDYNFSSILTSRVGGRSAIEDLLFNKVVTSGLGTVDQDYKSCS RNMAIADLVCSQYYNGIMVLPGVVDAEKMAMYTGSLTGAMVFGGLTAAAA
IPFATAVQARLNYVALQTNVLQENQKILAESFNQAVGNISLALSSVNDAI QQTSEALNTVAIAIKKIQTVVNQQGEALSHLTAQLSNNFQAISTSIQDIY
NRLEPPEANQQVDRLINGRLAALNAYVTQLLNQMSQIRQSRLLAQQKINE CVKSQSPRYGFCGNGTHIFSLTQTAPNGIFFMHAVLVPNKFTRVNASAGI
CVDNTRGYSLQPQLILYQFNNSWRVTPRNMYEPRLPRQADFIQLTDCSVT FYNTTAANLPNIIPDVIDVNQTVSDIIDNLPTATPPQWDVGIYNNTILNL
TVEINDLQERSKNLSQIADRLQNYIDNLNNTLVDLEWLNRVETGGYIPEA PRDGQAYVRKDGEWVLLSTF

In some embodiments, the recombinant SDCV S ectodomain trimer comprises protomers comprising the ectodomain sequence of SEQ ID NO: 42. In some embodiments, the recombinant SDCV S ectodomain trimer comprises protomers comprising residues 20-1093 of SEQ ID NO: 42 or residues 20-1120 of SEQ ID NO: 43. In some embodiments, the recombinant SDCV S ectodomain trimer comprises protomers comprising an ectodomain sequence at least 90% identical to the ectodomain sequence of SEQ ID NO: 39, wherein the SDCV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers. In some embodiments, the recombinant SDCV S ectodomain trimer comprises protomers comprising an amino acid sequence at least 90% identical to residues 20-1093 of SEQ ID NO: 39 or residues 20-1120 of SEQ ID NO: 43, wherein the SDCV S ectodomain trimer is stabilized in the prefusion conformation and comprises the "2P" substitution and/or modifications to remove the S1/S2 cleavage site and the S2' cleavage site of the protomers.

J. Single Chain S Proteins

In some embodiments, the CoV S ectodomain trimer can be composed of three single-chain CoV S ectodomain protomers, each including a single polypeptide chain including the 51 protein and S2 ectodomain. Single chain CoV S ectodomain protomers can be generated by mutating the S1/S2 and S2' protease cleavage sites to prevent cleavage and formation of distinct 51 and S2 polypeptide chains. In some embodiments, the 51 and S2 polypeptides in the single chain CoV S ectodomain protomers are joined by a linker, such as a peptide linker. Examples of peptide linkers that can be used include glycine, serine, and glycine-serine linkers. Any of the stabilizing mutations (or combinations thereof) disclosed herein can be included in the single chain coronavirus S ectodomain protomers as long as the coronavirus S ectodomain trimer composed of such protomers retains the desired properties (e.g., the prefusion conformation).

K. Linkage to a Trimerization Domain

In several embodiments, the S ectodomain protomers in the disclosed coronavirus S ectodomain trimers can be linked at their C-terminus (C-terminal linkage) to a trimerization domain to promote trimerization of the S ectodomain protomers, and to stabilize the membrane proximal aspect of the recombinant S ectodomains in a trimeric configuration.

Non-limiting examples of exogenous multimerization domains that promote stable trimers of soluble recombinant proteins include: the GCN4 leucine zipper (Harbury et al. 1993 Science 262:1401-1407), the trimerization motif from the lung surfactant protein (Hoppe et al. 1994 FEBS Lett 344:191-195), collagen (McAlinden et al. 2003 J Biol Chem 278:42200-42207), and the phage T4 fibritin Foldon (Miroshnikov et al. 1998 Protein Eng 11:329-414), any of which can be linked to a recombinant coronavirus S ectodomain described herein (e.g., by linkage to the C-terminus of S2) to promote trimerization of the recombinant coronavirus S ectodomain.

In some examples, the C-terminus of the S2 subunit of the S ectodomain can be linked to a T4 fibritin Foldon domain. In specific examples, the T4 fibritin Foldon domain can include the amino acid sequence GYIPEAPRDGQAYVRKDGEWVLLSTF (SEQ ID NO: 27), which adopts a .beta.-propeller conformation, and can fold and trimerize in an autonomous way (Tao et al. 1997 Structure 5:789-798). Optionally, the heterologous trimerization is connected to the recombinant coronavirus S ectodomain via a peptide linker, such as an amino acid linker. Non-limiting examples of peptide linkers that can be used include glycine, serine, and glycine-serine linkers.

L. Membrane Anchored Embodiments

In some embodiments, the coronavirus S ectodomain trimer can be membrane anchored, for example, for embodiments where the coronavirus S ectodomain trimer is expressed on an attenuated viral vaccine, or a virus like particle. In such embodiments, the protomers in the trimer typically each comprise a C-terminal linkage to a transmembrane domain, such as the transmembrane domain (and optionally the cytosolic tail) of corresponding coronavirus. For example, the protomers of a disclosed SARS-CoV S ectodomain trimer can be linked to a SARS-CoV S transmembrane and cytosolic tail. In some embodiments, one or more peptide linkers (such as a gly-ser linker, for example, a 10 amino acid glycine-serine peptide linker can be used to link the recombinant S ectodomain protomer to the transmembrane domain. The protomers linked to the

transmembrane domain can include any of the stabilizing mutations provided herein (or combinations thereof) as long as the recombinant coronavirus S ectodomain trimer formed from the protomers linked to the transmembrane domain retains the desired properties (e.g., the coronavirus S prefusion conformation).

M. Additional Description

The coronavirus S protein or fragments thereof can be produced using recombinant techniques, or chemically or enzymatically synthesized.

Analogs and variants of the coronavirus S protein or fragments thereof may be used in the methods and systems of the present invention. Through the use of recombinant DNA technology, variants of the coronavirus S protein or fragments thereof may be prepared by altering the underlying DNA. All such variations or alterations in the structure of the coronavirus S ectodomain or fragments thereof resulting in variants are included within the scope of this invention. Such variants include insertions, substitutions, or deletions of one or more amino acid residues, glycosylation variants, unglycosylated coronavirus S ectodomain or fragments thereof, organic and inorganic salts, covalently modified derivatives of the coronavirus S protein or fragments thereof, or a precursor thereof. Such variants may maintain one or more of the functional, biological activities of the coronavirus S protein or fragment thereof, such as binding to cell surface receptor. The coronavirus S protein or a fragment thereof can be modified, for example, by PEGylation, to increase the half-life of the protein in the recipient, to retard clearance from the pericardial space, and/or to make the protein more stable for delivery to a subject.

In some embodiments, a coronavirus S protein or fragment thereof useful within the disclosure is modified to produce peptide mimetics by replacement of one or more naturally occurring side chains of the 20 genetically encoded amino acids (or D-amino acids) with other side chains, for example with groups such as alkyl, lower alkyl, cyclic 4-, 5-, 6-, to 7-membered alkyl, amide, amide lower alkyl, amide di(lower alkyl), lower alkoxy, hydroxy, carboxy and the lower ester derivatives thereof, and with 4-, 5-, 6-, to 7-membered heterocyclics. For example, proline analogs can be made in which the ring size of the proline residue is changed from a 5-membered ring to a 4-, 6-, or 7-membered ring. Cyclic groups can be saturated or unsaturated, and if unsaturated, can be aromatic or non-aromatic. Heterocyclic groups can contain one or more nitrogen, oxygen, and/or sulphur heteroatoms. Examples of such groups include furazanyl, furyl, imidazolidinyl, imidazolyl, imidazolinyl, isothiazolyl, isoxazolyl, morpholinyl (e.g., morpholino), oxazolyl, piperazinyl (e.g., 1-piperazinyl), piperidyl (e.g., 1-piperidyl, piperidino), pyranyl, pyrazinyl, pyrazolidinyl, pyrazolinyl, pyrazolyl, pyridazinyl, pyridyl, pyrimidinyl, pyrrolidinyl (e.g., 1-pyrrolidinyl), pyrrolinyl, pyrrolyl, thiadiazolyl, thiazolyl, thienyl, thiomorpholinyl (e.g., thiomorpholino), and triazolyl groups. These heterocyclic groups can be substituted or unsubstituted. Where a group is substituted, the substituent can be alkyl, alkoxy, halogen, oxygen, or substituted or unsubstituted phenyl. Peptides, as well as peptide analogs and mimetics, can also be covalently bound to one or more of a variety of nonproteinaceous polymers, for example, polyethylene glycol, polypropylene glycol, or polyoxyalkenes, as described in U.S. Pat. Nos. 4,640,835; 4,496,668; 4,301,144; 4,668,417; 4,791,192; and 4,179,337.

N. Protein Nanoparticles

In some embodiments a protein nanoparticle is provided that includes one or more of the disclosed recombinant coronavirus S ectodomain trimers (e.g., a MERS-CoV S ectodomain trimer or a SARS-CoV S ectodomain trimer). Non-limiting example of nanoparticles include ferritin nanoparticles, encapsulin nanoparticles, Sulfur Oxygenase Reductase (SOR) nanoparticles, and lumazine synthase nanoparticles, which are comprised of an assembly of monomeric subunits including ferritin proteins, encapsulin proteins, SOR proteins, and lumazine synthase, respectively. Additional protein nanoparticle structures are described by Heinze et al., J Phys Chem B., 120(26):5945-52, 2016; Hsia et al., Nature, 535(7610):136-9, 2016; and King et al., Nature, 510(7503):103-8, 2014; each of which is incorporated by reference herein. To construct such protein nanoparticles a protomer of the coronavirus S ectodomain trimer can be linked to a subunit of the protein nanoparticle (such as a ferritin protein, an encapsulin protein, a SOR protein, or a lumazine synthase protein) and expressed in cells under appropriate conditions. The fusion protein self-assembles into a nanoparticle any can be purified.

In some embodiments, a protomer of a disclosed recombinant coronavirus S ectodomain trimer (e.g., a MERS-CoV S ectodomain trimer or a SARS-CoV S ectodomain trimer) can be linked to a ferritin subunit to construct a ferritin nanoparticle. Ferritin nanoparticles and their use for immunization purposes (e.g., for immunization against influenza antigens) have been disclosed in the art (see, e.g., Kanekiyo et al., Nature, 499:102-106, 2013, incorporated by reference herein in its entirety). Ferritin is a globular protein that is found in all animals, bacteria, and plants, and which acts primarily to control the rate and location of polynuclear Fe(III).sub.2O.sub.3 formation through the transportation of hydrated iron ions and protons to and from a mineralized core. The globular form of the ferritin nanoparticle is made up of monomeric subunits, which are polypeptides having a molecule weight of approximately 17-20 kDa. An example of the amino acid sequence of one such monomeric ferritin subunit is represented by:

TABLE-US-00037 (SEQ ID NO: 23) ESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWCYTHSLDGAGLFLF DHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHE QHISESINNIVDHAIKSKDHATFNFLQWYVAEQHEEEVLFKDILDKIELI GNENHGLYLADQYVKGIAKSRKS

Each monomeric subunit has the topology of a helix bundle which includes a four antiparallel helix motif, with a fifth shorter helix (the c-terminal helix) lying roughly perpendicular to the long axis of the 4 helix bundle. According to convention, the helices are labeled `A, B, C, D & E` from the N-terminus respectively. The N-terminal sequence lies adjacent to the capsid three-fold axis and extends to the surface, while the E helices pack together at the four-fold axis with the C-terminus extending into the capsid core. The consequence of this packing creates two pores on the capsid surface. It is expected that one or both of these pores represent the point by which the hydrated iron diffuses into and out of the capsid. Following production, these monomeric subunit proteins self-assemble into the globular ferritin protein. Thus, the globular form of ferritin comprises 24 monomeric, subunit proteins, and has a capsid-like structure having 432 symmetry. Methods of constructing ferritin nanoparticles are known to the person of ordinary skill in the art and are further described herein (see, e.g., Zhang, Int. J. Mol. Sci., 12:5406-5421, 2011, which is incorporated herein by reference in its entirety).

In specific examples, the ferritin polypeptide is E. coli ferritin, Helicobacter pylori ferritin, human light chain ferritin, bullfrog ferritin or a hybrid thereof, such as E. coli-human hybrid ferritin, E. coli-bullfrog hybrid ferritin, or human-bullfrog hybrid ferritin. Exemplary amino acid sequences of ferritin polypeptides and nucleic acid sequences encoding ferritin polypeptides for use to make a ferritin nanoparticle including a recombinant

coronavirus S ectodomain can be found in GENBANK.RTM., for example at accession numbers ZP_03085328, ZP_06990637, EJB64322.1, AAA35832, NP_000137 AAA49532, AAA49525, AAA49524 and AAA49523, which are specifically incorporated by reference herein in their entirety as available Apr. 10, 2015. In some embodiments, a recombinant coronavirus S ectodomain can be linked to a ferritin subunit including an amino acid sequence at least 80% (such as at least 85%, at least 90%, at least 95%, or at least 97%) identical to amino acid sequence set forth as SEQ ID NO: 122.

In some embodiments, a protomer of a disclosed recombinant coronavirus S ectodomain trimer (e.g., a MERS-CoV S ectodomain trimer or a SARS-CoV S ectodomain trimer) can be linked to a lumazine synthase subunit to construct a lumazine synthase nanoparticle. The globular form of lumazine synthase nanoparticle is made up of monomeric subunits; an example of the sequence of one such lumazine synthase subunit is provides as the amino acid sequence set forth as:

TABLE-US-00038 (SEQ ID NO: 24) MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDAIVRHGGREEDITL VRVPGSWEIPVAAGELARKEDIDAVIAIGVLIRGATPHFDYIASEVSKGL ADLSLELRKPITFGVITADTLEQAIERAGTKHGNKGWEAALSAIEMANLF KSLR.

In some embodiments, a protomer of a disclosed recombinant coronavirus S ectodomain trimer can be linked to a lumazine synthase subunit including an amino acid sequence at least 80% (such as at least 85%, at least 90%, at least 95%, or at least 97%) identical to amino acid sequence set forth as SEQ ID NO: 24.

In some embodiments, a protomer of a disclosed recombinant coronavirus S ectodomain trimer (e.g., a MERS-CoV S ectodomain trimer or a SARS-CoV S ectodomain trimer) can be linked to an encapsulin nanoparticle subunit to construct an encapsulin nanoparticle. The globular form of the encapsulin nanoparticle is made up of monomeric subunits; an example of the sequence of one such encapsulin subunit is provides as the amino acid sequence set forth as

TABLE-US-00039 (SEQ ID NO: 25) MEFLKRSFAPLTEKQWQEIDNRAREIFKTQLYGRKFVDVEGPYGWEYAAH PLGEVEVLSDENEVVKWGLRKSLPLIELRATFTLDLWELDNLERGKPNVD LSSLEETVRKVAEFEDEVIFRGCEKSGVKGLLSFEERKIECGSTPKDLLE AIVRALSIFSKDGIEGPYTLVINTDRWINFLKEEAGHYPLEKRVEECLRG GKIITTPRIEDALVVSERGGDFKLILGQDLSIGYEDREKDAVRLFITETF TFQVVNPEALILLKF.

In some embodiments, a protomer of a disclosed recombinant coronavirus S ectodomain trimer can be linked to an encapsulin subunit including an amino acid sequence at least 80% (such as at least 85%, at least 90%, at least 95%, or at least 97%) identical to amino acid sequence set forth as SEQ ID NO: 25.

Encapsulin proteins are a conserved family of bacterial proteins also known as linocin-like proteins that form large protein assemblies that function as a minimal compartment to package enzymes. The encapsulin assembly is made up of monomeric subunits, which are polypeptides having a molecule weight of approximately 30 kDa. Following production, the monomeric subunits self-assemble into the globular encapsulin assembly including 60, or in some cases, 180 monomeric subunits. Methods of constructing encapsulin nanoparticles are known to the person of ordinary skill in the art, and further described herein (see, for example, Sutter et al., Nature Struct. and Mol. Biol., 15:939-947, 2008, which is incorporated by reference herein in its entirety). In specific examples, the encapsulin polypeptide is bacterial encapsulin, such as Thermotoga maritime or Pyrococcus furiosus or Rhodococcus erythropolis or Myxococcus xanthus encapsulin.

In some embodiments, a protomer of a disclosed recombinant coronavirus S ectodomain trimer (e.g., a MERS-CoV S ectodomain trimer or a SARS-CoV S ectodomain trimer) can be linked to a Sulfur Oxygenase Reductase (SOR) subunit to construct a recombinant SOR nanoparticle. In some embodiments, the SOR subunit can include the amino acid sequence set forth as

TABLE-US-00040 (SEQ ID NO: 26) MEFLKRSFAPLTEKQWQEIDNRAREIFKTQLYGRKFVDVEGPYGWEYAAH PLGEVEVLSDENEVVKWGLRKSLPLIELRATFTLDLWELDNLERGKPNVD LSSLEETVRKVAEFEDEVIFRGCEKSGVKGLLSFEERKIECGSTPKDLLE AIVRALSIFSKDGIEGPYTLVINTDRWINFLKEEAGHYPLEKRVEECLRG GKIITTPRIEDALVVSERGGDFKLILGQDLSIGYEDREKDAVRLFITETF TFQVVNPEALILLKF.

In some embodiments, a protomer of a disclosed recombinant coronavirus S ectodomain trimer can be linked to a SOR subunit including an amino acid sequence at least 80% (such as at least 85%, at least 90%, at least 95%, or at least 97%) identical to amino acid sequence set forth as SEQ ID NO: 26.

SOR proteins are microbial proteins (for example from the thermoacidophilic archaeon Acidianus ambivalens that form 24 subunit protein assemblies. Methods of constructing SOR nanoparticles are known to the person of ordinary skill in the art (see, e.g., Urich et al., Science, 311:996-1000, 2006, which is incorporated by reference herein in its entirety). An example of an amino acid sequence of a SOR protein for use to make SOR nanoparticles is set forth in Urich et al., Science, 311:996-1000, 2006, which is incorporated by reference herein in its entirety.

For production purposes, the recombinant coronavirus S ectodomain linked to the nanoparticle subunit can include an N-terminal signal peptide that is cleaved during cellular processing. For example, the recombinant coronavirus S ectodomain protomer linked to the protein nanoparticle subunit can include a signal peptide at its N-terminus including, for example, a native coronavirus S signal peptide.

The protein nanoparticles can be expressed in appropriate cells (e.g., HEK 293 Freestyle cells) and fusion proteins are secreted from the cells self-assembled into nanoparticles. The nanoparticles can be purified using known techniques, for example by a few different chromatography procedures, e.g. Mono Q (anion exchange) followed by size exclusion (SUPEROSE.RTM. 6) chromatography.

Several embodiments include a monomeric subunit of a ferritin, encapsulin, SOR, or lumazine synthase protein, or any portion thereof which is capable of directing self-assembly of monomeric subunits into the globular form of the protein Amino acid sequences from monomeric subunits of any known ferritin, encapsulin, SOR, or lumazine synthase protein can be used to produce fusion proteins with the recombinant coronavirus S ectodomain or immunogenic fragment thereof, so long as the monomeric subunit is capable of self-assembling into a nanoparticle displaying the

recombinant coronavirus S ectodomain or immunogenic fragment thereof on its surface.

The fusion proteins need not comprise the full-length sequence of a monomeric subunit polypeptide of a ferritin, encapsulin, SOR, or lumazine synthase protein. Portions, or regions, of the monomeric subunit polypeptide can be utilized so long as the portion comprises amino acid sequences that direct self-assembly of monomeric subunits into the globular form of the protein.

III. Polynucleotides and Expression

Polynucleotides encoding a protomer of any of the disclosed recombinant S ectodomain trimers are also provided. These polynucleotides include DNA, cDNA and RNA sequences which encode the protomer, as well as vectors including the DNA, cDNA and RNA sequences, such as a DNA or RNA vector used for immunization. The genetic code to construct a variety of functionally equivalent nucleic acids, such as nucleic acids which differ in sequence but which encode the same protein sequence, or encode a conjugate or fusion protein including the nucleic acid sequence.

An exemplary nucleic acid sequence encoding MERS-CoV S protein is provided as SEQ ID NO: 5:

TABLE-US-00041 atgattcactccgtgttcctgctgatgttcctgctgactcctacagagag ctatgtggatgtgggacctgattccgtcaagagcgcctgcatcgaagtgg
acattcagcagaccttctttgataagacatggccaagacccatcgacgtg agcaaagccgat ggcatcatctaccctcaggggaggacctattccaatat
cacaattacttaccaggcctgttcccatatcagggagaccacggcgata tgtacgtgtattctgctggccatgcaacagggaccacacctcagaagctg
tttgtggctaactacagccaggacgtcaaacagttcgcaaatggatttgt ggtccgcatcggcgccgctgcaaactctaccggcacagtgatcatttcac
ctagcacttccgcaaccatccgaaaaatctacccagccttcatgctggga agctccgtgggcaattttagcgacgggaaaatgggacggttctttaacca
cacctggtgctgctgcctgatggatgcggcacactgctgagggctttct actgtatcctggagccacgcagcggaaaccactgccccgcaggaaatagc
tacacctcctttgccacatatcatactccagctaccgactgttccgatgg caactacaatcg aaacgcctctctgaataggtttcaaggaatacttcaacc
tgcggaattgcacattcatgtacacttataacatcaccgaggacgaaatt ctggagtggttcggaatcactcagaccgcacagggcgtgcacctgttttc
tagtcgctacgtcgacctgtatggcgggaacatgttccagttt tgccactc tgcccgtgtacgatacccatcaagtactattccatcattcctcattcaatc
cgcagcattcagtccgatcgaaaggcttgggccgctttctacgtgtataa actgcagccact gaccttcctgctggactttagcgtcgatggctacatcc
ggagagccattgactgcgggtttaatgatctgtcccagctgcactgttct tacgaaagtttcgacgtggagtccggcgtgtattctgtctcaagctttga
ggccaagccctctgggagtgtggtcgagcaggctgaaggagtggagtgcg atttcagtcctctgctgtcagggaccccccctcaggtgtacaacttcaag
cggctggtctttactaactgtaactacaatctgaccaagctgctgtcact gttcagcgtgaatgactttacatgctcccagatcagccccgcagccatt g
ctagtaactgttactcctctctgatcctggactacttctcatatccactg agtatgaagag cgacctgagcgtgagttcagccggccccatcagccagtt
caactataaacagagcttcagcaatcctacat gcctgattctggctactg tgccacataatctgactaccatcactaagcccctgaaatactcctatatt
aacaagtgcagccggttcctgtccgacgatagaaccgaagtgccacagct ggtcaacgcca atcagtactctccctgtgtgagtatcgtccttcaaccg
tgtgggaagacggggattactatagaaaacagctgagccccctggaggga ggaggatggctggtggcatccggatctacagtcgccatgactgagcagct
gcagatggggttcggaatcacagtgcagtacggcacagacactaactctg tctgtcccaag ctggaattcgctaacgatactaagatcgcaagtcagctg
ggaaactgcgtggagtactctctgtatggcgtgagtggcagaggggtctt ccagaattgtaccgcagtgggcgtccgacagcagcggtttgtgtacgacg
cctatcagaatctggtcggctactatagcgacgatgggaactactattgc ctgagggcctgtgtgagcgtccctgtgtccgtcatctacgataaggaaac
caaaacacacgccacactgttcgggtccgtggcttgcgagcatattagct ccacaatgtctcagtacagtagatcaactaggtcaatgctgaagaggcgc
gatagcacctatggacctctgcagacaccagtggggtgtgtcctgggact ggtgaactctagtctgtttgtcgaggactgcaagctgccccttgggccaga
gcctgtgcgccctgcccgacaccccagcacctgacccccctggaggcgtg cggagcgtgcccggcgagatgcggctggccagcatcgccttcaaccaccc
catccaggtggaccagctgaacagcagctacttcaagctgagcatcccca ccaacttcagct tcggcgtgacccaggagtacatccagaccaccatccag
aaggtgaccgtggactgcaagcagtacgtgtgcaacggcttccagaagtg cgagcagctgctgcgggagtacggccagttctgcagcaagatcaaccagg
ccctgcacggcgccaacctgcggcaggacgacagcgtgcggaacctgttc gccagcgtgaagagcagccagagcagccccatcatccccggcttcggcgg
cgacttcaacctgacctgctggagccgtgagcatcagcaccggcagcc ggagcgcccggagcgccatcgaggacctgctgttcgacaaggtgaccatc
gccgaccccggctacatgcagggctacgacgactgcatgcagcagggccc cgccagcgc ccgggacctgatctgcgcccagtacgtggccggctacaagg
tgctgccccccctgatggacgtgaacatggaggccgcctacaccagcagc ctgctgggcag catcgccggcgtgggctggaccgccggcctgagcagctt
cgccgccatcccctcgcccagagcatcttctaccggctgaacggcgtgg gcatcacccagcaggtgctgagcgagaaccagaagctgatcgccaacaag
ttcaaccaggccctgggcgccatgcagaccggcttcaccaccaccaacga ggccttccacaa ggtgcaggacgccgtgaacaacaacgcccaggccctga
gcaagctggccagcgagctgagcaacaccttcggcgccatcagcgccagc atcggcgacat catccagcggctggacgtgctggagcaggacgcccagat
cgaccggctgatcaacggccggctgaccaccctgaacgccttcgtggccc agcagctgg tgcggagcgagagcgccgccctgagcgcccagctggccaag
gacaaggtgaacgagtgcgtgaaggcccagagcaagcggagcggcttctg cggccagggcacccacatcgtgagcttcgtggtgaacgccccaacggcc
tgtacttcatgcacgtgggctactacccagcaaccacatcgaggtgg tg agcgctacggcctgtgcgacgccgccaaccccaccaactgcatcgcccc
cgtgaacggctacttcatcaagaccaacaacaccccggatcgtggacgagt ggagctacaccggcagcagcttctacgccccgagcccatcaccagcctg
aacaccaagtacgtggcccccaggtgacctaccagaacatcagcaccaa cctgcccccc ccctgctgggcaacagcaccggcatcgacttccaggacg
agctggacgagttcttcaagaacgtgagcaccagcatccccaacttcggc agcctgaccca gatcaacaccaccctgctggacctgacctacgagatgct
gagcctgcagcaggtggtgaaggccctgaacgagagctacatcgacctga aggagctgggcaactacacctactacaacaagtggcccctggtacatctgg
ctgggcttcatcgccggcctggtggccctggccctgtgcgtgttcttcat cctgtgctgcaccggctgcggcaccaactgcatgggcaagctgaagtgca
accggtgctgcgaccggtacgaggagtacgacctggagcccacaaggtg cacgtgcact ga

The DNA sequence of the MERS-CoV S protomer provided above can be modified to introduce the amino acid substitutions and deletions disclosed herein for prefusion stabilization, such as the "2P" substitutions.

In several embodiments, the nucleic acid molecule encodes a precursor of the protomer, that, when expressed in an appropriate cell, is processed into a disclosed coronavirus S ectodomain protomer that can self-assemble into the corresponding recombinant coronavirus S ectodomain trimer. For example, the nucleic acid molecule can encode a recombinant coronavirus S ectodomain including a N-terminal signal sequence for entry into the cellular secretory system that is proteolytically cleaved in the during processing of the recombinant coronavirus S ectodomain in the cell.

In several embodiments, the nucleic acid molecule encodes a precursor S polypeptide that, when expressed in an appropriate cell, is processed into a disclosed recombinant coronavirus S ectodomain protomer including S1 and S2 polypeptides, wherein the recombinant S ectodomain protomer includes any of the appropriate stabilizing modifications described herein, and optionally can be linked to a trimerization domain, such as a T4 Fibritin trimerization domain.

Exemplary nucleic acids can be prepared by cloning techniques. Examples of appropriate cloning and sequencing techniques, and instructions sufficient to direct persons of skill through many cloning exercises are known (see, e.g., Sambrook et al. (Molecular Cloning: A Laboratory Manual, 4.sup.th ed, Cold Spring Harbor, N.Y., 2012) and Ausubel et al. (In Current Protocols in Molecular Biology, John Wiley & Sons, New York, through supplement 104, 2013).

Nucleic acids can also be prepared by amplification methods. Amplification methods include polymerase chain reaction (PCR), the ligase chain reaction (LCR), the transcription-based amplification system (TAS), the self-sustained sequence replication system (3SR). A wide variety of cloning methods, host cells, and in vitro amplification methodologies are well known to persons of skill.

The polynucleotides encoding a disclosed recombinant coronavirus S ectodomain protomer can include a recombinant DNA which is incorporated into a vector (such as an expression vector) into an autonomously replicating plasmid or virus or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (such as a cDNA) independent of other sequences. The nucleotides can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single and double forms of DNA.

Polynucleotide sequences encoding a disclosed recombinant coronavirus S ectodomain protomer can be operatively linked to expression control sequences. An expression control sequence operatively linked to a coding sequence is ligated such that expression of the coding sequence is achieved under conditions compatible with the expression control sequences. The expression control sequences include, but are not limited to, appropriate promoters, enhancers, transcription terminators, a start codon (i.e., ATG) in front of a protein-encoding gene, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons.

DNA sequences encoding the disclosed recombinant S ectodomain protomer can be expressed in vitro by DNA transfer into a suitable host cell. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art. Non-limiting examples of suitable host cells include bacteria, archea, insect, fungi (for example, yeast), plant, and animal cells (for example, mammalian cells, such as human). Exemplary cells of use include Escherichia coli, Bacillus subtilis, Saccharomyces cerevisiae, Salmonella typhimurium, SF9 cells, C129 cells, 293 cells, Neurospora, and immortalized mammalian myeloid and lymphoid cell lines. Techniques for the propagation of mammalian cells in culture are well-known (see, e.g., Helgason and Miller (Eds.), 2012, Basic Cell Culture Protocols (Methods in Molecular Biology), 4.sup.th Ed., Humana Press). Examples of commonly used mammalian host cell lines are VERO and HeLa cells, CHO cells, and WI38, BHK, and COS cell lines, although cell lines may be used, such as cells designed to provide higher expression, desirable glycosylation patterns, or other features. In some embodiments, the host cells include HEK293 cells or derivatives thereof, such as GnTI.sup.-/- cells (ATCC.RTM. No. CRL-3022), or HEK-293F cells.

Transformation of a host cell with recombinant DNA can be carried out by conventional techniques. Where the host is prokaryotic, such as, but not limited to, E. coli, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the CaCl.sub.2) method using standard procedures. Alternatively, MgCl.sub.2 or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell if desired, or by electroporation.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate coprecipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or viral vectors can be used. Eukaryotic cells can also be co-transformed with polynucleotide sequences encoding a disclosed antigen, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein (see for example, Viral Expression Vectors, Springer press, Muzyczka ed., 2011). Appropriate expression systems such as plasmids and vectors of use in producing proteins in cells including higher eukaryotic cells such as the COS, CHO, HeLa and myeloma cell lines.

In one non-limiting example, a disclosed immunogen is expressed using the pVRC8400 vector (described in Barouch et al., J. Virol., 79, 8828-8834, 2005, which is incorporated by reference herein). Modifications can be made to a nucleic acid encoding a disclosed recombinant coronavirus S ectodomain protomer without diminishing its biological activity. Some modifications can be made to facilitate the cloning, expression, or incorporation of the targeting molecule into a fusion protein. Such modifications are well known to those of skill in the art and include, for example, termination codons, a methionine added at the amino terminus to provide an initiation, site, additional amino acids placed on either terminus to create conveniently located restriction sites, or additional amino acids (such as poly His) to aid in purification steps.

In some embodiments, the disclosed recombinant coronavirus S ectodomain protomer can be expressed in cells under conditions where the recombinant coronavirus S ectodomain protomer can self-assemble into trimers which are secreted from the cells into the cell media. In such embodiments, each recombinant coronavirus S ectodomain protomer contains a leader sequence (signal peptide) that causes the protein to enter the secretory system, where the signal peptide is cleaved and the protomers form a trimer, before being secreted in the cell media. The medium can be centrifuged and recombinant coronavirus S ectodomain trimer purified from the supernatant.

IV. Viral Vectors

A nucleic acid molecule encoding a protomer of a disclosed recombinant coronavirus S ectodomain trimer can be included in a viral vector, for example, for expression of the immunogen in a host cell, or for immunization of a subject as disclosed herein. In some embodiments, the viral vectors are administered to a subject as part of a prime-boost vaccination. In several embodiments, the viral vectors are included in a vaccine, such as a primer vaccine or a booster vaccine for use in a prime-boost vaccination.

In several examples, the viral vector can be replication-competent. For example, the viral vector can have a mutation in the viral genome that does not inhibit viral replication in host cells. The viral vector also can be conditionally replication-competent. In other examples, the viral vector is replication-deficient in host cells.

A number of viral vectors have been constructed, that can be used to express the disclosed antigens, including polyoma, i.e., SV40 (Madzak et al., 1992, J. Gen. Virol., 73:15331536), adenovirus (Berkner, 1992, Cur. Top. Microbiol. Immunol., 158:39-6; Berliner et al., 1988, Bio Techniques, 6:616-629; Gorziglia et al., 1992, J. Virol., 66:4407-4412; Quantin et al., 1992, Proc. Natl. Acad. Sci. USA, 89:2581-2584; Rosenfeld et al., 1992, Cell, 68:143-155; Wilkinson et al., 1992, Nucl. Acids Res., 20:2233-2239; Stratford-Perricaudet et al., 1990, Hum. Gene Ther., 1:241-256), vaccinia virus (Mackett et al., 1992, Biotechnology, 24:495-499), adeno-associated virus (Muzyczka, 1992, Curr. Top. Microbiol. Immunol., 158:91-123; On et al., 1990, Gene, 89:279-282), herpes viruses including HSV and EBV (Margolskee, 1992, Curr. Top. Microbiol. Immunol., 158:67-90; Johnson et al., 1992, J. Virol., 66:29522965; Fink et al., 1992, Hum. Gene Ther. 3:11-19; Breakfield et al., 1987, Mol. Neurobiol., 1:337-371; Fresse et al., 1990, Biochem. Pharmacol., 40:2189-2199), Sindbis viruses (H. Herweijer et al., 1995, Human Gene Therapy 6:1161-1167; U.S. Pat. Nos. 5,091,309 and 5,2217,879), alphaviruses (S. Schlesinger, 1993, Trends Biotechnol. 11:18-22; I. Frolov et al., 1996, Proc. Natl. Acad. Sci. USA 93:11371-11377) and retroviruses of avian (Brandyopadhyay et al., 1984, Mol. Cell Biol., 4:749-754; Petropouplos et al., 1992, J. Virol., 66:3391-3397), murine (Miller, 1992, Curr. Top. Microbiol. Immunol., 158:1-24; Miller et al., 1985, Mol. Cell Biol., 5:431-437; Sorge et al., 1984, Mol. Cell Biol., 4:1730-1737; Mann et al., 1985, J. Virol., 54:401-407), and human origin (Page et al., 1990, J. Virol., 64:5370-5276; Buchschalcher et al., 1992, J. Virol., 66:2731-2739). Baculovirus (Autographa californica multinuclear polyhedrosis virus; AcMNPV) vectors are also known in the art, and may be obtained from commercial sources (such as PharMingen, San Diego, Calif.; Protein Sciences Corp., Meriden, Conn.; Stratagene, La Jolla, Calif.).

In several embodiments, the viral vector can include an adenoviral vector that expresses a protomer of a disclosed recombinant coronavirus S ectodomain trimer. Adenovirus from various origins, subtypes, or mixture of subtypes can be used as the source of the viral genome for the adenoviral vector. Non-human adenovirus (e.g., simian, chimpanzee, gorilla, avian, canine, ovine, or bovine adenoviruses) can be used to generate the adenoviral vector. For example, a simian adenovirus can be used as the source of the viral genome of the adenoviral vector. A simian adenovirus can be of serotype 1, 3, 7, 11, 16, 18, 19, 20, 27, 33, 38, 39, 48, 49, 50, or any other simian adenoviral serotype. A simian adenovirus can be referred to by using any suitable abbreviation known in the art, such as, for example, SV, SAdV, SAV or sAV. In some examples, a simian adenoviral vector is a simian adenoviral vector of serotype 3, 7, 11, 16, 18, 19, 20, 27, 33, 38, or 39. In one example, a chimpanzee serotype C Ad3 vector is used (see, e.g., Peruzzi et al., Vaccine, 27:1293-1300, 2009). Human adenovirus can be used as the source of the viral genome for the adenoviral vector. Human adenovirus can be of various subgroups or serotypes. For instance, an adenovirus can be of subgroup A (e.g., serotypes 12, 18, and 31), subgroup B (e.g., serotypes 3, 7, 11, 14, 16, 21, 34, 35, and 50), subgroup C (e.g., serotypes 1, 2, 5, and 6), subgroup D (e.g., serotypes 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 36-39, and 42-48), subgroup E (e.g., serotype 4), subgroup F (e.g., serotypes 40 and 41), an unclassified serogroup (e.g., serotypes 49 and 51), or any other adenoviral serotype. The person of ordinary skill in the art is familiar with replication competent and deficient adenoviral vectors (including singly and multiply replication deficient adenoviral vectors). Examples of replication-deficient adenoviral vectors, including multiply replication-deficient adenoviral vectors, are disclosed in U.S. Pat. Nos. 5,837,511; 5,851,806; 5,994,106; 6,127,175; 6,482,616; and 7,195,896, and International Patent Application Nos. WO 94/28152, WO 95/02697, WO 95/16772, WO 95/34671, WO 96/22378, WO 97/12986, WO 97/21826, and WO 03/02231 1.

V. Virus-Like Particles

In some embodiments, a virus-like particle (VLP) is provided that includes a disclosed recombinant coronavirus S ectodomain trimer. Typically such VLPs include a recombinant coronavirus S ectodomain trimer that is membrane anchored by a C-terminal transmembrane domain, for example the recombinant coronavirus S ectodomain protomers in the trimer each can be linked to a transmembrane domain and cytosolic tail from the corresponding coronavirus. VLPs lack the viral components that are required for virus replication and thus represent a highly attenuated, replication-incompetent form of a virus. However, the VLP can display a polypeptide (e.g., a recombinant coronavirus S ectodomain trimer) that is analogous to that expressed on infectious virus particles and can eliciting an immune response to the corresponding coronavirus when administered to a subject. Virus like particles and methods of their production are known and familiar to the person of ordinary skill in the art, and viral proteins from several viruses are known to form VLPs, including human papillomavirus, HIV (Kang et al., Biol. Chem. 380: 353-64 (1999)), Semliki-Forest virus (Notka et al., Biol. Chem. 380: 341-52 (1999)), human polyomavirus (Goldmann et al., J. Virol. 73: 4465-9 (1999)), rotavirus (Jiang et al., Vaccine 17: 1005-13 (1999)), parvovirus (Casal, Biotechnology and Applied Biochemistry, Vol 29, Part 2, pp 141-150 (1999)), canine parvovirus (Hurtado et al., J. Virol. 70: 5422-9 (1996)), hepatitis E virus (Li et al., J. Virol. 71: 7207-13 (1997)), and Newcastle disease virus. The formation of such VLPs can be detected by any suitable technique. Examples of suitable techniques known in the art for detection of VLPs in a medium include, e.g., electron microscopy techniques, dynamic light scattering (DLS), selective chromatographic separation (e.g., ion exchange, hydrophobic interaction, and/or size exclusion chromatographic separation of the VLPs) and density gradient centrifugation.

VI. Immunogenic Compositions

Immunogenic compositions comprising a disclosed immunogen (e.g., a disclosed recombinant coronavirus S ectodomain trimer or nucleic acid molecule encoding a protomer of disclosed recombinant coronavirus S ectodomain trimer) and a pharmaceutically acceptable carrier are also provided. Such pharmaceutical compositions can be administered to subjects by a variety of administration modes known to the person of ordinary skill in the art, for example, intramuscular, intradermal, subcutaneous, intravenous, intra-arterial, intra-articular, intraperitoneal, intranasal, sublingual, tonsillar, oropharyngeal, or other parenteral and mucosal routes. In several embodiments, pharmaceutical compositions including one or more of the disclosed immunogens are immunogenic compositions. Actual methods for preparing administrable compositions will be known or apparent to those skilled in the art and are described in more detail in such publications as Remingtons Pharmaceutical Sciences, 19.sup.th Ed., Mack Publishing Company, Easton, Pa., 1995.

Thus, an immunogen described herein can be formulated with pharmaceutically acceptable carriers to help retain biological activity while also promoting increased stability during storage within an acceptable temperature range. Potential carriers include, but are not limited to, physiologically balanced culture medium, phosphate buffer saline solution, water, emulsions (e.g., oil/water or water/oil emulsions), various types of wetting agents, cryoprotective additives or stabilizers such as proteins, peptides or hydrolysates (e.g., albumin, gelatin), sugars (e.g., sucrose, lactose, sorbitol), amino acids (e.g., sodium glutamate), or other protective agents. The resulting aqueous solutions may be packaged for use as is or lyophilized. Lyophilized

preparations are combined with a sterile solution prior to administration for either single or multiple dosing.

Formulated compositions, especially liquid formulations, may contain a bacteriostat to prevent or minimize degradation during storage, including but not limited to effective concentrations (usually 1% w/v) of benzyl alcohol, phenol, m-cresol, chlorobutanol, methylparaben, and/or propylparaben. A bacteriostat may be contraindicated for some patients; therefore, a lyophilized formulation may be reconstituted in a solution either containing or not containing such a component.

The immunogenic compositions of the disclosure can contain as pharmaceutically acceptable vehicles substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, and triethanolamine oleate. The immunogenic composition may optionally include an adjuvant to enhance an immune response of the host. Suitable adjuvants are, for example, toll-like receptor agonists, alum, AlPO4, alhydrogel, Lipid-A and derivatives or variants thereof, oil-emulsions, saponins, neutral liposomes, liposomes containing the vaccine and cytokines, non-ionic block copolymers, and chemokines. Non-ionic block polymers containing polyoxyethylene (POE) and polyxylpropylene (POP), such as POE-POP-POE block copolymers, MPL.TM. (3-O-deacylated monophosphoryl lipid A; Corixa, Hamilton, Ind.) and IL-12 (Genetics Institute, Cambridge, Mass.), among many other suitable adjuvants well known in the art, may be used as an adjuvant (Newman et al., 1998, Critical Reviews in Therapeutic Drug Carrier Systems 15:89-142). These adjuvants have the advantage in that they help to stimulate the immune system in a non-specific way, thus enhancing the immune response to a pharmaceutical product.

In some instances it may be desirable to combine a disclosed immunogen, with other pharmaceutical products (e.g., vaccines) which induce protective responses to other agents. For example, a composition including a recombinant paramyxovirus as described herein can be can be administered simultaneously (typically separately) or sequentially with other vaccines recommended by the Advisory Committee on Immunization Practices (ACIP; cdc.gov/vaccines/acip/index.html) for the targeted age group (e.g., infants from approximately one to six months of age), such as an influenza vaccine or a varicella zoster vaccine. As such, a disclosed immunogen including a recombinant coronavirus S ectodomain trimer described herein may be administered simultaneously or sequentially with vaccines against, for example, hepatitis B (HepB), diphtheria, tetanus and pertussis (DTaP), pneumococcal bacteria (PCV), Haemophilus influenzae type b (Hib), polio, influenza and rotavirus.

In some embodiments, the composition can be provided as a sterile composition. The pharmaceutical composition typically contains an effective amount of a disclosed immunogen and can be prepared by conventional techniques. Typically, the amount of immunogen in each dose of the immunogenic composition is selected as an amount which induces an immune response without significant, adverse side effects. In some embodiments, the composition can be provided in unit dosage form for use to induce an immune response in a subject. A unit dosage form contains a suitable single preselected dosage for administration to a subject, or suitable marked or measured multiples of two or more preselected unit dosages, and/or a metering mechanism for administering the unit dose or multiples thereof. In other embodiments, the composition further includes an adjuvant.

VII. Methods of Inducing an Immune Response

The disclosed immunogens (e.g., recombinant coronavirus S ectodomain trimer, a nucleic acid molecule (such as an RNA molecule) or vector encoding a protomer of a disclosed recombinant coronavirus S ectodomain trimer, or a protein nanoparticle or virus like particle comprising a disclosed recombinant coronavirus S ectodomain trimer) can be administered to a subject to induce an immune response to the corresponding coronavirus S ectodomain in the subject. In a particular example, the subject is a human. The immune response can be a protective immune response, for example a response that inhibits subsequent infection with the corresponding coronavirus. Elicitation of the immune response can also be used to treat or inhibit infection and illnesses associated with the corresponding coronavirus.

A subject can be selected for treatment that has, or is at risk for developing infection with the coronavirus corresponding to the S protein in the immunogen, for example because of exposure or the possibility of exposure to the coronavirus. Following administration of a disclosed immunogen, the subject can be monitored for infection or symptoms associated with the coronavirus, or both.

Typical subjects intended for treatment with the therapeutics and methods of the present disclosure include humans, as well as non-human primates and other animals. To identify subjects for prophylaxis or treatment according to the methods of the disclosure, accepted screening methods are employed to determine risk factors associated with a targeted or suspected disease or condition, or to determine the status of an existing disease or condition in a subject. These screening methods include, for example, conventional work-ups to determine environmental, familial, occupational, and other such risk factors that may be associated with the targeted or suspected disease or condition, as well as diagnostic methods, such as various ELISA and other immunoassay methods to detect and/or characterize coronavirus infection. These and other routine methods allow the clinician to select patients in need of therapy using the methods and pharmaceutical compositions of the disclosure. In accordance with these methods and principles, a composition can be administered according to the teachings herein, or other conventional methods, as an independent prophylaxis or treatment program, or as a follow-up, adjunct or coordinate treatment regimen to other treatments.

The administration of a disclosed immunogen can be for prophylactic or therapeutic purpose. When provided prophylactically, the disclosed therapeutic agents are provided in advance of any symptom, for example, in advance of infection. The prophylactic administration of the disclosed therapeutic agents serves to prevent or ameliorate any subsequent infection. When provided therapeutically, the disclosed therapeutic agents are provided at or after the onset of a symptom of disease or infection, for example, after development of a symptom of infection with the coronavirus corresponding to the S protein in the immunogen, or after diagnosis with the coronavirus infection. The therapeutic agents can thus be provided prior to the anticipated exposure to the coronavirus so as to attenuate the anticipated severity, duration or extent of an infection and/or associated disease symptoms, after exposure or suspected exposure to the virus, or after the actual initiation of an infection.

The immunogens described herein, and immunogenic compositions thereof, are provided to a subject in an amount effective to induce or enhance an immune response against the coronavirus S protein in the immunogen in the subject, preferably a human. The actual dosage of disclosed immunogen will vary according to factors such as the disease indication and particular status of the subject (for example, the subject's age, size, fitness, extent of symptoms, susceptibility factors, and the like), time and route of administration, other drugs or treatments being administered concurrently, as well as

the specific pharmacology o f the composition for eliciting the desired activity or biological response in the subject. Dosage regimens can be adjusted to provide an optimum prophylactic or therapeutic response.

An immunogenic composition including one or more of the disclosed immunogens can be used in coordinate (or prime-boost) vaccination protocols or combinatorial formulations. In certain embodiments, novel combinatorial immunogenic compositions and coordinate immunization protocols employ separate immunogens or formulations, each directed toward eliciting an anti-viral immune response, such as an immune response to coronavirus S proteins. Separate immunogenic compositions that elicit the anti-viral immune response can be combined in a polyvalent immunogenic composition administered to a subject in a single immunization step, or they can be administered separately (in monovalent immunogenic compositions) in a coordinate (or prime-boost) immunization protocol.

There can be several boosts, and each boost can be a different disclosed immunogen. In some examples that the boost may be the same immunogen as another boost, or the prime. The prime and boost can be administered as a single dose or multiple doses, for example two doses, three doses, four doses, five doses, six doses or more can be administered to a subject over days, weeks or months. Multiple boosts can also be given, such one to five (e.g., 1, 2, 3, 4 or 5 boosts), or more. Different dosages can be used in a series of sequential immunizations. For example a relatively large dose in a primary immunization and then a boost with relatively smaller doses.

In some embodiments, the boost can be administered about two, about three to eight, or about four, weeks following the prime, or about several months after the prime. In some embodiments, the boost can be administered about 5, about 6, about 7, about 8, about 10, about 12, about 18, about 24, months after the prime, or more or less time after the prime. Periodic additional boosts can also be used at appropriate time points to enhance the subject's "immune memory." The adequacy of the vaccination parameters chosen, e.g., formulation, dose, regimen and the like, can be determined by taking aliquots of serum from the subject and assaying antibody titers during the course of the immunization program. In addition, the clinical condition of the subject can be monitored for the desired effect, e.g., prevention of infection or improvement in disease state (e.g., reduction in viral load). If such monitoring indicates that vaccination is sub-optimal, the subject can be boosted with an additional dose of immunogenic composition, and the vaccination parameters can be modified in a fashion expected to potentiate the immune response.

In some embodiments, the prime-boost method can include DNA-primer and protein-boost vaccination protocol to a subject. The method can include two or more administrations of the nucleic acid molecule or the protein.

For protein therapeutics, typically, each human dose will comprise 1-1000 .mu.g of protein, such as from about 1 .mu.g to about 100 .mu.g, for example, from about 1 .mu.g to about 50 .mu.g, such as about 1 .mu.g, about 2 .mu.g, about 5 .mu.g, about 10 .mu.g, about 15 .mu.g, about 20 .mu.g, about 25 .mu.g, about 30 .mu.g, about 40 .mu.g, or about 50 .mu.g.

The amount utilized in an immunogenic composition is selected based on the subject population (e.g., infant or elderly). An optimal amount for a particular composition can be ascertained by standard studies involving observation of antibody titers and other responses in subjects. It is understood that a therapeutically effective amount of a disclosed immunogen, such as a disclosed recombinant coronavirus S ectodomain trimer, viral vector, or nucleic acid molecule in a immunogenic composition, can include an amount that is ineffective at eliciting an immune response by administration of a single dose, but that is effective upon administration of multiple dosages, for example in a prime-boost administration protocol.

Upon administration of a disclosed immunogen of this disclosure, the immune system of the subject typically responds to the immunogenic composition by producing antibodies specific for the coronavirus S ectodomain trimer included in the immunogen. Such a response signifies that an immunologically effective dose was delivered to the subject.

In some embodiments, the antibody response of a subject will be determined in the context of evaluating effective dosages/immunization protocols. In most instances it will be sufficient to assess the antibody titer in serum or plasma obtained from the subject. Decisions as to whether to administer booster inoculations and/or to change the amount of the therapeutic agent administered to the individual can be at least partially based on the antibody titer level. The antibody titer level can be based on, for example, an immunobinding assay which measures the concentration of antibodies in the serum which bind to an antigen including, for example, the recombinant coronavirus S ectodomain trimer included in the immunogen.

Coronavirus infection does not need to be completely eliminated or reduced or prevented for the methods to be effective. For example, elicitation of an immune response to a coronavirus with one or more of the disclosed immunogens can reduce or inhibit infection with the coronavirus by a desired amount, for example, by at least 10%, at least 20%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even at least 100% (elimination or prevention of detectable infected cells), as compared to infection with the coronavirus in the absence of the immunogen. In additional examples, coronavirus replication can be reduced or inhibited by the disclosed methods. Coronavirus replication does not need to be completely eliminated for the method to be effective. For example, the immune response elicited using one or more of the disclosed immunogens can reduce replication of the corresponding coronavirus by a desired amount, for example, by at least 10%, at least 20%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, or even at least 100% (elimination or prevention of detectable replication of the coronavirus), as compared to replication of the coronavirus in the absence of the immune response.

In some embodiments, the disclosed immunogen is administered to the subject simultaneously with the administration of the adjuvant. In other embodiments, the disclosed immunogen is administered to the subject after the administration of the adjuvant and within a sufficient amount of time to induce the immune response.

One approach to administration of nucleic acids is direct immunization with plasmid DNA, such as with a mammalian expression plasmid. Immunization by nucleic acid constructs is well known in the art and taught, for example, in U.S. Pat. No. 5,643,578 (which describes methods of immunizing vertebrates by introducing DNA encoding a desired antigen to elicit a cell-mediated or a humoral response), and U.S. Pat. Nos. 5,593,972 and 5,817,637 (which describe operably linking a nucleic acid sequence encoding an antigen to regulatory sequences enabling expression). U.S. Pat. No. 5,880,103 describes several methods of delivery of nucleic acids encoding immunogenic peptides or other antigens to an organism. The methods include liposomal delivery of the nucleic acids (or of the synthetic peptides themselves), and immune-stimulating constructs, or ISCOMS.TM., negatively charged cage-like structures of 30-40 nm in size formed spontaneously on mixing cholesterol and Quil A.TM. (saponin).

Protective immunity has been generated in a variety of experimental models of infection, including toxoplasmosis and Epstein-Barr virus-induced tumors, using ISCOMS.TM. as the delivery vehicle for antigens (Mowat and Donachie, Immunol. Today 12:383, 1991). Doses of antigen as low as 1 .mu.g encapsulated in ISCOMS.TM. have been found to produce Class I mediated CTL responses (Takahashi et al., Nature 344:873, 1990).

In some embodiments, a plasmid DNA vaccine is used to express a disclosed immunogen in a subject. For example, a nucleic acid molecule encoding a disclosed immunogen can be administered to a subject to induce an immune response to the coronavirus S protein included in the immunogen. In some embodiments, the nucleic acid molecule can be included on a plasmid vector for DNA immunization, such as the pVRC8400 vector (described in Barouch et al., J. Virol, 79, 8828-8834, 2005, which is incorporated by reference herein).

In another approach to using nucleic acids for immunization, a disclosed recombinant coronavirus S ectodomain or recombinant coronavirus S ectodomain trimer can be expressed by attenuated viral hosts or vectors or bacterial vectors. Recombinant vaccinia virus, adeno-associated virus (AAV), herpes virus, retrovirus, cytogmeglo virus or other viral vectors can be used to express the peptide or protein, thereby eliciting a CTL response. For example, vaccinia vectors and methods useful in immunization protocols are described in U.S. Pat. No. 4,722,848. BCG (Bacillus Calmette Guerin) provides another vector for expression of the peptides (see Stover, Nature 351:456-460, 1991).

In one embodiment, a nucleic acid encoding a disclosed recombinant coronavirus S ectodomain or coronavirus S ectodomain trimer is introduced directly into cells. For example, the nucleic acid can be loaded onto gold microspheres by standard methods and introduced into the skin by a device such as Bio-Rad's HELIOS.TM. Gene Gun. The nucleic acids can be "naked," consisting of plasmids under control of a strong promoter. Typically, the DNA is injected into muscle, although it can also be injected directly into other sites. Dosages for injection are usually around 0.5 .mu.g/kg to about 50 mg/kg, and typically are about 0.005 mg/kg to about 5 mg/kg (see, e.g., U.S. Pat. No. 5,589,466).

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In another embodiment, an mRNA-based immunization protocol can be used to deliver a nucleic acid encoding a disclosed recombinant coronavirus S ectodomain or coronavirus S ectodomain trimer directly into cells. In some embodiments, nucleic acid-based vaccines based on mRNA may provide a potent alternative to the previously mentioned approaches. mRNA vaccines preclude safety concerns about DNA integration into the host genome and can be directly translated in the host cell cytoplasm. Moreover, the simple cell-free, in vitro synthesis of RNA avoids the manufacturing complications associated with viral vectors. Two exemplary forms of RNA-based vaccination that can be used to deliver a nucleic acid encoding a disclosed recombinant coronavirus S ectodomain or coronavirus S ectodomain trimer include conventional non-amplifying mRNA immunization (see, e.g., Petsch et al., "Protective efficacy of in vitro synthesized, specific mRNA vaccines against influenza A virus infection," Nature biotechnology, 30(12):1210-6, 2012) and self-amplifying mRNA immunization (see, e.g., Geall et al., "Nonviral delivery of self-amplifying RNA vaccines," PNAS, 109(36): 14604-14609, 2012; Magini et al., "Self-Amplifying mRNA Vaccines Expressing Multiple Conserved Influenza Antigens Confer Protection against Homologous and Heterosubtypic Viral Challenge," PLoS One, 11(8):e0161193, 2016; and Brito et al., "Self-amplifying mRNA vaccines," Adv Genet., 89:179-233, 2015).

In some embodiments, administration of a therapeutically effective amount of one or more of the disclosed immunogens to a subject induces a neutralizing immune response in the subject. To assess neutralization activity, following immunization of a subject, serum can be collected from the subject at appropriate time points, frozen, and stored for neutralization testing. Methods to assay for neutralization activity are known to the person of ordinary skill in the art and are further described herein, and include, but are not limited to, plaque reduction neutralization (PRNT) assays, microneutralization assays, flow cytometry based assays, single-cycle infection assays. In some embodiments, the serum neutralization activity can be assayed using a panel of coronavirus pseudoviruses. For example, to test the immunogenicity of the vaccine candidates against multiple MERS-CoV strains--without the requirement of a biosafety level 3 facility--a pseudotyped reporter virus neutralization assay was previously developed (Wand et al., Nat Commun, 6:7712, 2015), similar to that previously developed for SARS-CoV (Martin et al., Vaccine 26, 6338, 2008; Yang et al., Nature 428, 561, 2004; Naldini et al., PNAS 93, 11382, 1996; Yang et al., PNAS 102, 797, 2005).

In some embodiments, administration of a therapeutically effective amount of one or more of the disclosed immunogens to a subject induces a neutralizing immune response in the subject. To assess neutralization activity, following immunization of a subject, serum can be collected from the subject at appropriate time points, frozen, and stored for neutralization testing. Methods to assay for neutralization activity are known to the person of ordinary skill in the art and are further described herein, and include, but are not limited to, plaque reduction neutralization (PRNT) assays, microneutralization assays, flow cytometry based assays, single-cycle infection assays. In some embodiments, the serum neutralization activity can be assayed using a panel of coronavirus pseudoviruses.

EXAMPLES

The following examples are provided to illustrate particular features of certain embodiments, but the scope of the claims should not be limited to those features exemplified.

Example 1

Prefusion Stabilized MERS-CoV S Protein

This example describes development of a recombinant MERS-CoV S ectodomain trimer that is stabilized in a prefusion conformation.

The structure of the prefusion S ectodomain trimer of a human betacoronavirus was recently elucidated (Kirchdoerfer et al., "Prefusion structure of a human coronavirus spike protein," Nature, 351:118-121, 2016). This structure was further investigated to reveal several key details about human coronavirus spike architecture. First, receptor-binding elements within S1 cap the fusion-mediating elements in S2, likely preventing their

conformational rearrangement (FIG. 1) until triggering occurs.

The S1 C-terminal domains appear interdigitated and form extensive quaternary interactions (FIG. 2A), suggesting conformational flexibility or "breathing" would be required for the HKU1-CoV Spike to make receptor interactions similar to those made between the SARS-CoV receptor binding domain (RBD) and ACE2 receptors (FIG. 2A). In addition, the structure revealed two sub-domains, SD-1 and SD-2, in HKU1-CoV S1 (FIGS. 2C, 2D). The SD-2 loop contains the site dedicated to HKU1-CoV S furin cleavage; and furin cleavage at the S1/S2 junction is a process necessary for infection (FIG. 2D). S2 contains four classical components of a Class 1 fusion machine: a fusion peptide (FIG. 3A), two heptad repeats, HR1 (FIG. 3B) and HR2, and a transmembrane domain.

Structure-based stabilization of betacoronavirus prefusion trimers. The HKU1-CoV prefusion S structure was used as a starting point to design mutations that would stabilize betacoronavirus S trimers in the prefusion conformation. Dozens of possible stabilizing mutations were designed and tested in the context of the MERS-CoV S protein. Two mutations were identified to be particularly effective for stabilizing the MERS-CoV S protein in its prefusion conformation: V1060P and L1061P (or their combination) (FIG. 4). MERS-CoV S proteins including these mutations also had >50 fold increased expression (FIG. 4). These two proline substitutions are located at the top portion (membrane distal) of the MERS-CoV S2 central helix and HR1 to prevent pre-to-postfusion conformational changes. Prefusion stabilization of the MERS-CoV S protein is preliminarily indicated by increased expression levels when these mutations are combined compared to an S2 truncated, but otherwise wild-type (WT) MERS-CoV S (C6) (FIGS. 4B,4C). WT MERS-CoV S likely spontaneously flips from pre-to-postfusion conformation. Corresponding double proline mutations in SARS-CoV and HKU1-CoV S also increased expression above WT S.

S protein immunogens were expressed from codon-optimized genes encoding the S ectodomain (without TM and CT) with a C-terminal T4 fibritin trimerization domain, an HRV3C cleavage site, a 6.times.His-tag and a Twin-Strep-tag that were cloned into the eukaryotic-expression vector paH. Following sequence verification, expression plasmids were transiently transfected into FreeStyle293 cells. Three hours after transfection, kifunensine was added to a final concentration of 5 .mu.M. Cultures were harvested six days later, and secreted protein was purified from the supernatant and soluble protein w purified from the supernatant by passage over Ni.sup.2+-NTA and StrepTactin resin using affinity tags on the C-terminus of the proteins. The purified proteins were then be passed over a size-exclusion column to assess their oligomeric state and to isolate monodisperse fractions corresponding to trimeric ectodomains. Protein expression levels were then assessed by SDS-PAGE (10 .mu.L of protein-bound resin was boiled and loaded per lane). This expression strategy was used to generated generate and test proline-substituted variants of MERS-CoV S (Eng1 strain, residues 1-1291), SARS-CoV S (Tort strain, residues 1-1190) and HCoV-HKU1 S (N5 strain, residues 1-1276). The MERS-CoV S ectodomain trimers included a 748-RSVR-751 (residues 748-751 of SEQ ID NO: 1) to 748-ASVG-751 (residues 748-751 of SEQ ID NO: 3) substitutions to remove the S1/S2 cleavage site.

Mice (N=5/group) were vaccinated with 0.1 .mu.g, 1 .mu.g, or 10 .mu.g of the MERS-CoV S trimer stabilized in the prefusion conformation by V1060P and L1061P substitutions to evaluate the effectiveness of the resulting immune response (FIG. 5). As a comparison, mice were also vaccinated with the MERS-CoV S1 protein, which was previously found to induce robust neutralizing antibody responses associated with protection, and MERS-CoV S ectodomain trimers with WT sequence. Control mice were given PBS. The immunogens were based on the England1 ("Eng") MERS-CoV strain.

Immunizations were performed as weeks 0 and 3. Two weeks following the last immunization, serum was collected and tested for neutralization against various MERS pseudovirus strains: England1, Florida USA2, Bisha1, Korea002, JordanN3, Buraidah1, and Indiana USA1. Serum was diluted, in triplicate, and incubated with MERS-CoV pseudovirus prior to inoculation of Huh7.5 cells. Dilution curves were fitted to mock cells and cells exposed to un-neutralized virus as 100% and 0% neutralization, respectively. IC90 titers were calculated as the dilution of serum needed to neutralize 90% of MERS-CoV pseudovirus.

Vaccination with the MERS-CoV S1, wild-type MERS-CoV S ectodomain, or the prefusion stabilized MERS-CoV S ectodomain induced similar robust levels of neutralizing antibodies against homologous MERS-CoV England1 reporter pseudovirus at dosages of 10 .mu.g, but the prefusion-stabilized spike was superior at lower dosages (FIG. 5A). Further, when tested against homologous virus strains, the prefusion stabilized MERS-CoV ectodomain trimer produced a superior immune response (FIG. 5B).

Additional assays were performed to show that vaccination with MERS S-2P ectodomain trimer elicited more non-RBD binding antibodies than MERS S1 ectodomain trimer (FIG. 6A), and higher levels of neutralizing activity targeting a greater diversity of epitopes than antigens based on RBD or S1 monomer (FIG. 6B).

Additionally, challenge studies were performed to determine if the prefusion-stabilized MERS-CoV S ectodomain trimer could prevent MERS-CoV infection in an animal model (FIG. 7). The challenge studies were performed using C57BL/6J mice that were genetically engineered using CRISPR-Cas9 genomic editing to encode human DPP4 mutations (288L and 330R; "288/330.sup.+/+") as previously described (see, Cockrell et al., "A mouse model for MERS coronavirus-induced acute respiratory distress syndrome." Nature Microbiology. 2:16226, 2016, which is incorporated by reference herein). These mice are known to be susceptible to invention with MERS-CoV. The 288/330.sup.+/+ mice were vaccinated with 0.1 .mu.g MERS CoV-S ectodomain timer with the double proline mutation using the Sigma Adjuvant System at weeks 0 and 3. Four weeks following final vaccination, the mice were challenged with a lethal dose of mouse-adapted MERS virus and monitored for survival and weight loss. As shown in FIG. 7, prior immunization with the prefusion stabilized MERS-CoV S ectodomain trimer protected against lethal MERS challenge in mice.

Example 2

Prefusion Stabilized Coronavirus Spike Proteins

HKU1-CoV is closely related to other betacoronaviruses, such as the zoonotic viruses SARS-CoV and MERS-CoV, both of which are associated with high mortality. Accordingly, additional coronavirus S ectodomain trimers stabilized in the prefusion conformation by double proline mutations at the HR1/central helix junction were evaluated as vaccine candidates.

Due to the structural similarity of coronavirus S proteins, the sequences of these proteins can be readily aligned to identify structural domains, such as the HR1 and central helix. FIG. 8 illustrates the structural domains of the HKU1, SARS, and MERS-CoV S proteins, as well as positioning of double proline substitutions to stabilize these proteins in the prefusion conformation. FIG. 8 shows a sequence alignment of the S2 subunit of the HKU1-CoV, SARS-CoV, MERS-CoV, HKU9-CoV, NL63-CoV, and 229E-CoV S proteins, showing relevant sequence homology. The HR1 spans the .alpha.13, .alpha.14, .alpha.17, and .alpha.16 helixes, including approximately residues 996-1064 (relative to HKU1-CoV numbering shown in the figure). The central helix is the .alpha.17 helix, including approximately residues 1068-1110. The HR2 includes approximates residues 1245-1276 (relative to HKU1-CoV numbering shown in the figure). The transmembrane domain begins at approximately residue 1292 (relative to HKU1-CoV numbering shown in the figure).

Proline substitutions were introduced into the SARS-CoV, HKU1-CoV, OC43-CoV, HKU9-CoV, WIV1-CoV, MHV-CoV, NL63-CoV and 229E-CoV. The SARS-CoV substitutions were K968P, V969P, or K968P and V969P. The HKU1-CoV substitutions are N1067P, L1068P, or N1067P and L1068P. The OC43-CoV substitutions are A1079P, L1080P, or A1079P and L1080P. The HKU9-CoV substitutions are G983P, L984P, or G983P and L984P. The WIV1-CoV substitutions are K969P, V970P, or K969P and V970P. The MHV-CoV substitutions are A1073P, L1074P, or A1073P and L1074P. The NL63-CoV substitutions are S1052P, I1053P, or S1052P and I1053P. The 229E-CoV substitutions are I869P, I870P, or I869P and I870P. Soluble SARS-CoV, HKU1-CoV, OC43-CoV, HKU9-CoV, WIV1-CoV, MHV-CoV, NL63-CoV and 229E-CoV S ectodomain trimers containing the indicated mutations, a signal peptide, and a C-terminal linkage to a T4 Fibritin trimerization domain and streptavidin tag were expressed in cells and purified as described in Example 1. Including the signal peptide and T4 Fibritin trimerization domain, protomer sequences of the referenced ectodomain trimers including the double proline substitutions are as follows:

SARS-CoV S 2P (K968P and V969P, SEQ ID NO: 30)

HKU1-CoV S 2P (N1067P and L1068P, SEQ ID NO: 31)

HKU9-CoV S 2P (G983P and L984P, SEQ ID NO: 32)

OC43-CoV S 2P (A1079P and L1080P, SEQ ID NO: 33)

WIV1-CoV S 2P (K969P and V970P, SEQ ID NO: 34)

MHV-CoV S 2P (A1073P and L1074P, SEQ ID NO: 35)

NL63-CoV S 2P (51052P and I1053P, SEQ ID NO: 36)

229E-CoV S 2P (I869P and I870P, SEQ ID NO: 37)

PEDV-CoV S 2P (I1076P and L1077P, SEQ ID NO: 40)

As shown in FIG. 10, the proline substitutions boosted the expression of the SARS-CoV and HKU1-CoV S ectodomains.

The thermal stability of the wild-type SARS-CoV S ectodomain (SARS-S-WT) and SARS-CoV S ectodomain with K968P and V969P (SARS-S-2P) was assessed (FIG. 11). About 3 .mu.g SARS-S-WT or SARS-S-2P samples in TBS buffer (2 mM Tris pH8.0, 200 mM NaCl) were incubated at different temperature for 1 hour. The samples were then analyzed on the NativePAGE Novex Bis-Tris gels (Invitrogen) using procedures suggested by the manufacturer. As shown in FIG. 11, the SARS-S-2P has higher thermal stability than SARS-S-WT.

The expressed protein trimers were further analyzed by gel chromatography. FIG. 12 illustrates results from chromatography experiments concerning wild-type SARS-CoV S ectodomain (SARS-S-WT), SARS-CoV S ectodomain with K968P and V969P (SARS-S-2P), wild-type MERS-CoV S ectodomain (MERS-S-WT), MERS-CoV S ectodomain with V1060P and L1061P (MERS-S-2P), wild-type HKU1-CoV S ectodomain (HKU1-S-WT), HKU1-CoV S ectodomain with N1067P and L1068P (HKU1-S-2P). In all three cases, a larger peak was observed for the double proline mutant, show a many-fold increase in expression of the double proline mutant relative to the WT ectodomain trimer.

The conformation of the double proline mutant SARS-CoV, HKU1-CoV, and MERS-CoV S variants was assessed by negative stain electron microscopy (FIG. 13A). In each case the S variants with the double proline mutant were homogeneous and form trimers in the expected prefusion shape. Each of these ectodomain trimers was purified as a sing le peak and formed trimers in the typical prefusion conformation. In contrast, corresponding S proteins with native sequences formed trimers of mixed conformation, with some trimers in the typical prefusion conformation and others in the typical elongated post-fusion conformation.

Additionally, the conformation of the double proline mutant OC43-CoV, WIV1-CoV, and PEDV-CoV, and 229E-CoV S variants was also assessed by negative stain electron microscopy (FIGS. 13B-13C). In each case the S variants with the double proline mutant were homogeneous and form trimers in the expected prefusion shape. Each of these ectodomain trimers was purified as a single peak and formed trimers in the typical prefusion conformation.

When low resolution negative stain reconstructions of S trimer constructs from HKU1-CoV (FIG. 14A), MERS-CoV (FIG. 14B), SARS-CoV (FIG. 14C), OC43-CoV S 2P (FIG. 14D), WIV1-CoV S 2P (FIG. 14E), PEDV-CoV S 2P (FIG. 14F), and 229E S-2P (FIG. 14G) were reconstructed from the EM data, the articles all formed homogeneous trimeric spike protein structures.

To assess the immunogenicity of the SARS-CoV S 2P ectodomain trimer, mice (N=5/group) were vaccinated with 0.1 .mu.g or 1 .mu.g of the SARS-CoV S trimer stabilized in the prefusion conformation by K968P and V969P substitutions (SEQ ID NO: 30) to evaluate the effectiveness of the resulting immune response (FIG. 15). As a comparison, mice were also vaccinated with the SARS-CoV S ectodomain trimers with WT sequence. The immunogens were based on the TOR2 SARS-CoV strain. Immunizations were performed as weeks 0 and 3. Two weeks following the last

immunization, serum was collected and tested for neutralization against autologous SARS pseudovirus. Serum was diluted, in triplicate, and incubated with SARS-CoV pseudovirus prior to inoculation of Huh7.5 cells. Dilution curves were fitted to mock cells and cells exposed to un-neutralized virus as 100% and 0% neutralization, respectively. IC90 titers were calculated as the dilution of serum needed to neutralize 90% of SARS-CoV pseudovirus. As shown in FIG. 15, vaccination with the prefusion stabilized SARS-CoV S ectodomain induced a superior immune response relative to the wild-type SARS-CoV S ectodomain, particularly at the 0.1 .mu.g dose.

Additionally, mice (N=5/group) were vaccinated with 0.1 .mu.g, 1 .mu.g, or 10 .mu.g of the HKU1-CoV S trimer stabilized in the prefusion conformation by N1067P and L1068P substitutions (SEQ ID NO: 31) to evaluate the effectiveness of the resulting immune response (FIG. 15). As a comparison, mice were also vaccinated with the HKU1-CoV S ectodomain trimers with WT sequence. Immunizations were performed as weeks 0 and 3. Two weeks following the last immunization, serum was collected and tested for neutralization against autologous HKU1-CoV pseudovirus. Serum was diluted, in triplicate, and incubated with HKU1-CoV pseudovirus prior to inoculation of Huh7.5 cells. Dilution curves were fitted to mock cells and cells exposed to un-neutralized virus as 100% and 0% neutralization, respectively. IC90 titers were calculated as the dilution of serum needed to neutralize 90% of HKU1-CoV pseudovirus. As shown in FIG. 15, vaccination with the prefusion stabilized HKU1-CoV S ectodomain induced a superior immune response relative to the wild-type HKU1-CoV S ectodomain, particularly at the 0.1 .mu.g dose.

In additional assays, mice (N=5/group) were vaccinated with 1 .mu.g of the OC43-CoV S ectodomain trimer stabilized in the prefusion conformation by A1079P and L1080P substitutions (SEQ ID NO: 33) or with 1 .mu.g of the WIV1-CoV S ectodomain trimer stabilized in the prefusion conformation by K969P and V970P substitutions (SEQ ID NO: 34) to evaluate the effectiveness of the resulting immune response (FIG. 16). PBS was used as a control. Immunizations were performed as weeks 0 and 3. Two weeks following the last immunization, serum was collected and tested for binding to the corresponding immunogen by ELISA. As shown in FIG. 16, vaccination with the prefusion stabilized OC43-CoV S ectodomain trimer or the prefusion stabilized WIV1-CoV S ectodomain trimer elicited antibodies that target the corresponding ectodomain trimers.

It will be apparent that the precise details of the methods or compositions described may be varied or modified without departing from the spirit of the described embodiments. We claim all such modifications and variations that fall within the scope and spirit of the claims below.

[Questions submitted for the record and the responses by Dr. Socal follow:]

May 13, 2021

Dr. Mariana Socal, MD PhD MS MPP
Associate Scientist
Johns Hopkins Bloomberg School of Public Health
624 North Broadway, Suite 301
Baltimore, MD 21224

Dear Dr. Socal:

I would like to thank you for testifying at the May 5, 2021 Subcommittee on Health, Employment, Labor, and Pensions hearing entitled "*Lower Drug Costs Now: Expanding Access to Affordable Health Care.*"

Please find enclosed additional questions submitted by Committee members following the hearing. Please provide a written response no later than Thursday, May 20, 2021, for inclusion in the official hearing record. Your responses should be sent to Mariah Mowbray and Daniel Foster of the Committee staff. They can be contacted at 202-225-3725 should you have any questions.

I appreciate your time and continued contribution to the work of the Committee.

Sincerely,

ROBERT C. "BOBBY" SCOTT
Chairman

Enclosure

Subcommittee on Health, Employment, Labor, and Pensions Hearing
"Lower Drug Costs Now: Expanding Access to Affordable Health Care"
Wednesday, May 5, 2021
12:00 p.m. (Eastern Time)

Representative Mark DeSaulnier (D – CA)

1. Dr. Socal, in response to a question posed to you by Chairman Scott, you said that 93% of the most frequently prescribed drugs in America were developed in part by NIH-funded research. Given the clear benefit that drug companies get from government-funded research, do you think it would be beneficial to study the appropriate profit margins companies that use government research to develop their drug should be able to make? It seems to me that it could be one of the ways we can lower the cost of drugs to consumers and the cost of health care to employers.

Representative Diana Harshbarger (R – TN)

1. Dr. Socal, you mentioned that U.S. drug prices are not offset by manufacturer rebates and price before rebates determine American's cost-sharing amount. Dr. Holtz-Eakin mentioned the business model PBMs have built around revenues tied to the amount in rebates they can secure from drugmakers. **In your opinion, what purpose do manufacturer rebates serve and should the U.S. continue to allow or endorse the practice of PBMs "negotiating" rebates on behalf of health plans and patients if they have no effect on reducing drug costs and could possibly be causing greater escalation of drug prices to the end payers and patients?**

2. Dr. Socal and Mr. Mitchell both mentioned the need for transparency as an opportunity for reducing patients' cost share. Dr. Socal specifically mentioned if employer-sponsored plans had access to federally-negotiated drug prices, they could offer lower prices to their workers. Yet, the Pharmaceutical Care Management Association (PCMA) — the trade and lobbying association for PBMs — has rigorously fought transparency, saying if Americans had access to that kind of information it would cause drug prices to further increase. **Dr. Socal, how do you reconcile suggesting PBMs could further negotiate prices down from the federally-negotiated price when they currently fight tooth and nail to hide this information behind claims of "proprietary and trade secrets"?**

JOHNS HOPKINS
BLOOMBERG SCHOOL
of PUBLIC HEALTH

Baltimore, May 24, 2021

To: Mr. ROBERT C. "BOBBY" SCOTT
Chairman
Committee on Education and Labor
U.S. House Of Representatives
2176 Rayburn House Office Building
Washington, DC 20515-6100

Re: Subcommittee on Health, Employment, Labor, and Pensions Hearing:
"Lower Drug Costs Now: Expanding Access to Affordable Health Care"
Wednesday, May 5, 2021, 12:00 p.m.

Dear Chairman Scott,

Thanks so much for the opportunity of serving as a witness in the hearing "Lower Drug Costs Now: Expanding Access to Affordable Health Care." I also would like to thank Rep. DeSaulnier and Rep. Harshbarger for sending important questions, which I received with great interest. Please find my answers below.

Please do not hesitate to let me know if you have any questions or concerns.

Sincerely,

Mariana Socal, MD MS MPP PhD
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Representative Mark DeSaulnier (D – CA)

1. Dr. Socal, in response to a question posed to you by Chairman Scott, you said that 93% of the most frequently prescribed drugs in America were developed in part by NIH-funded research. Given the clear benefit that drug companies get from government-funded research, do you think it would be beneficial to study the appropriate profit margins companies that use government research to develop their drug should be able to make? It seems to me that it could be one of the ways we can lower the cost of drugs to consumers and the cost of health care to employers.

Thank you so much for this important question. Yes, it would be important to study the appropriate profit margins that pharmaceutical manufacturers who use government-funded research in developing their drugs should be able to make on these products. A large scientific literature shows that most new drugs originate as scientific breakthroughs from research funded by the federal government, though its agencies such as the National Institutes of Health (NIH).

The analysis that I cited in my testimony was a 2017 study[1] that found that 97% of all new drugs approved by the FDA from 2010 to 2016 had received NIH support for the identification of the drug or its mechanistic basis, and 93% of the 100 most commonly prescribed drugs in the US had received NIH support. Sometimes the federal government's investment is on the drug itself, but it can also be on the identification of potential mechanisms of action, molecular targets, etc. Government funding is especially critical at the initial phases of drug development when failure rates are very high.

This analysis is important because, even though the government – funded by the taxpayer – is providing support to the development of so many drugs, when the drug is finally available and commercialized, only the final drug manufacturer gets reimbursed for their investment. Not only the government does not get reimbursed for their investment, but also the taxpayers get charged egregiously high prices to access the very drugs whose research they helped finance.

Representative Diana Harshbarger (R – TN)

1. Dr. Socal, you mentioned that U.S. drug prices are not offset by manufacturer rebates and price before rebates determine American's cost-sharing amount. Dr. Holtz-Eakin mentioned the business model PBMs have built around revenues tied to the amount in rebates they can secure from drug makers. In your opinion, what purpose do manufacturer rebates serve and should the U.S. continue to allow or endorse the practice of PBMs "negotiating" rebates on behalf of health plans and patients if they have no effect on reducing drug costs and could possibly be causing greater escalation of drug prices to the end payers and patients?

Thank you so much for this important question. Drug rebates currently serve the purpose of helping PBMs (pharmacy benefit managers) negotiate drug prices on behalf of America's self-insured employers as well as private insurers, Medicare prescription drug plans, and others. But whether rebates actually bring drug costs down is a matter of debate. Drug manufacturers set drug prices in the United States. Manufacturers may set the drug's price at whichever level they

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[1] Griesenauer RH, Moore R, Kinch MS. NIH Support for FDA-Approved Medicines.
Cell Chemical Biology Volume 24, ISSUE 11, P1315-1316, November 16, 2017. https://www.cell.com/cell-chemical-biology/fulltext/S2451-9456(17)30397-5

want and may increase the drug's price at any time and without any specific reason. The price set by the manufacturer is called the “list price,” and it is essentially a 'sticker' price that serves as the basis for negotiations. A drug's net or final price can only be known once all rebates and discounts have been accounted for. This typically happens a long time after the drug was dispensed to the patient. The final net price of a drug is not publicly known.

There are two problems with using rebates as a means to negotiate prices. First, the PBM is the only entity that has full information on the amount of rebate that was negotiated. Rebates are never disclosed to the public and often are not disclosed to insurers either. Therefore, relying on drug rebates for drug price negotiations lacks transparency and adds complexity to the market, often making it impossible to know how much a drug truly costs. It also makes it difficult for self-insured employers to monitor the activities of the PBM and hold them accountable.

Second, because PBMs are allowed to keep a portion of the negotiated rebate as revenue, PBMs may prefer to cover drugs that are more expensive but can offer greater rebates – at the expense of cheaper drugs that do not offer rebates because they are already of lower cost. This model incentivizes branded drug manufacturers to progressively increase their drugs’ list prices so that they can provide progressively larger rebates to the PBMs and maintain favorable coverage in the drug formularies that PBMs design. Thus, it can be argued that the use of rebates to negotiate drugs prices has contributed for the growth in those very prices that it aims to control. Insulin drug manufacturers have supported this correlation in testimonies presented at a hearing carried out by the House of Representatives' Committee of Energy and Commerce on April 2019.[2]

Because of the incentives to provide ever-increasing rebates, US drug list prices are now too high. This is a problem because patients who are uninsured, who are in the deductible phase of a high-deductible-health plan, or who are insured but their insurer doesn’t cover the drug that they need may pay the full list price in order to access their drug. In addition, patients who must pay coinsurance – a percentage of the drug’s cost – in order to obtain their drugs often pay a percentage of the list price.

Getting rid of rebates does not necessarily mean that list prices would go down. If list prices they weren’t to decrease, overall drug spending might rise. But it is likely that list prices would continue to be negotiated and, in the long term, drug spending would decrease as well.

2. Dr. Socal and Mr. Mitchell both mentioned the need for transparency as an opportunity for reducing patients' cost share. Dr. Socal specifically mentioned if employer-sponsored plans had access to federally-negotiated drug prices, they could offer lower prices to their workers. Yet, the Pharmaceutical Care Management Association (PCMA) — the trade and lobbying association for PBMs — has rigorously fought transparency, saying if Americans had access to that kind of information it would cause drug prices to further increase. Dr. Socal, how do you reconcile suggesting PBMs could further negotiate prices down from the federally-negotiated price when they currently fight tooth and nail to hide this information behind claims of "proprietary and trade secrets"?

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[2] House Committee on Energy and Commerce. Hearing on “Priced Out of a Lifesaving Drug: Getting Answers on the Rising Cost of Insulin.” Wednesday, April 10, 2019 - 10:30am. https://energycommerce.house.gov/committee-activity/hearings/hearing-on-priced-out-of-a-lifesaving-drug-getting-answers-on-the-rising

The model proposed in HR3 would have federally-negotiated prices for drugs without competition. For these drugs, the PBM model of negotiation is currently not successful, because without competition these manufacturers do not have an incentive to offer large rebates or discounts on their drugs. The lack of transparency benefits PBMs because it prevents self-insured employers from understanding the drug's final price. It also benefits PBMs because it makes it easier for PBMs to keep a portion of the price concessions that they negotiate as revenue.

In a transparent system, PBM accountability to self-insured employers would increase. With a transparent price benchmark available, self-insured employers could easily distinguish whether the prices offered to them by the PBM represent a "good deal" or not. Furthermore, in this model the PBM would be free to continue to negotiate the drug's price, pushing down drug costs beyond the federally-negotiated price.

[Questions submitted for the record and the responses by Mr. Mitchell follow:]

May 13, 2021

David Mitchell
Founder and President
Patients For Affordable Drugs Now
7820 Hampden Lane
Bethesda, MD 20814

Dear Mr. Mitchell:

I would like to thank you for testifying at the May 5, 2021 Subcommittee on Health, Employment, Labor, and Pensions hearing entitled "*Lower Drug Costs Now: Expanding Access to Affordable Health Care.*"

Please find enclosed additional questions submitted by Committee members following the hearing. Please provide a written response no later than Thursday, May 20, 2021, for inclusion in the official hearing record. Your responses should be sent to Mariah Mowbray and Daniel Foster of the Committee staff. They can be contacted at 202-225-3725 should you have any questions.

I appreciate your time and continued contribution to the work of the Committee.

Sincerely,

ROBERT C. "BOBBY" SCOTT
Chairman

Enclosure

Subcommittee on Health, Employment, Labor, and Pensions Hearing
"Lower Drug Costs Now: Expanding Access to Affordable Health Care"
Wednesday, May 5, 2021
12:00 p.m. (Eastern Time)

Representative Haley Stevens (D – MI)

1. Mr. Mitchell, through your prior experience as a small business owner, could you please discuss the challenges you faced in affording health coverage at your company and how drug prices contribute to this problem for businesses in this country?

Representative Diana Harshbarger (R – TN)

1. Mr. Mitchell, how do Pharmacy Benefit Managers (PBMs) impact patient costs and access to pharmacies?

2. Mr. Mitchell, how might reforms in the PBM industry and greater transparency itself lead to lowering prices, and perhaps level the playing field for independent community pharmacies?

(And if not, [to the above question]), What specific polices would do so?

3. Mr. Mitchell, in December, 2020 the U.S. Supreme Court unanimously ruled in *Rutledge v. PCMA* that ERISA does not preempt an Arkansas state law that regulates reimbursement levels paid by PBMs to pharmacies and that other states can follow suit. What are your thoughts on possible federal legislation, similar to Arkansas' state law, to ensure PBMs reimburse pharmacies at least at a price equal to the pharmacies' acquisition costs so that pharmacies can stay open for rural and underserved communities, to avoid pharmacy deserts where we need health care providers the most to ensure health equity in this country?

David Mitchell Responses to Questions for the Record
Subcommittee on Health, Employment, Labor, and Pensions Hearing
"Lower Drug Costs Now: Expanding Access to Affordable Health Care"
Wednesday, May 5, 2021

Representative Haley Stevens (D – MI)

Mr. Mitchell, through your prior experience as a small business owner, could you please discuss the challenges you faced in affording health coverage at your company and how drug prices contribute to this problem for businesses in this country?

For more than 30 years, I helped start and build a small business. Health care always figured prominently in our benefits package to attract and retain talent. As drug costs rose, they became more of a factor in our premiums and benefit structure. I knew that as an employer, I had to either absorb the costs, shift costs to my employees, or take money out of paychecks in order to cover the increases. Increasing costs meant that we spent more of our total compensation dollar on health care—including prescription drug coverage—and so had less money for paychecks and other benefits.

Small business owners are desperate for relief—most say that providing health coverage is the biggest challenge they face. When asked about the root cause of rising costs of healthcare, 93% of business owners say they think pharmaceutical companies bear responsibility.[1] Two in three said the drug pricing system needs major overhaul and 77% said they would support government negotiation as a solution. H.R. 3 would provide enormous relief to small business owners and allow them to put money back in the paychecks of their employees.

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[1] Survey: Small Business Owners Eager for Relief from High Healthcare Costs, Say Bringing Down Costs Should be a Top Priority. March 2021. https://irp-cdn.multiscreensite.com/b4559992/files/uploaded/%20SBAF%20Healthcare%20Survey%20March%202021.pdf

Representative Diana Harshbarger (R – TN):

1. **Mr. Mitchell, how do Pharmacy Benefit Managers (PBMs) impact patient costs and access to pharmacies?**

While the headwaters of our drug pricing problems are the list prices set by drug corporations, there are other reforms needed in the supply chain. Pharmacy benefit managers (PBMs) undoubtedly contribute to increased costs to patients and the health care system. As the middlemen between insurance companies and drug manufacturers, PBMs make deals with drugmakers that determine which drugs are placed on formularies and which drugs get preferential placement on these formularies, leading to greater use.

The intent of this framework was that PBMs would use formularies to negotiate discounts that would improve healthy drug choices and with savings that could be passed onto patients and payers. Instead, patients like me don't know if the preferred drug on a PBM formulary is there because it is the best drug, because it is the least expensive drug among equally effective options, or because the PBM got a large rebate from the manufacturer. Without transparency, it is impossible to know how much of a rebate is going to the PBM, to the insurer, to lower my premiums, or to reduce my out-of-pocket costs at the pharmacy counter. We need transparency to ensure PBMs are operating in the best interests of those they are supposed to serve — patients and consumers.

2. **Mr. Mitchell, how might reforms in the PBM industry and greater transparency itself lead to lowering prices, and perhaps level the playing field for independent community pharmacies?**

I am not familiar with the specific policy changes being advocated by independent pharmacists. Our focus is on patients. I do know that pharmacies often don't know when they buy and stock a drug how much they will actually be paid for that drug by the PBM six months later. That is an absurd policy by any measure and makes it difficult for independently-owned pharmacies to make financial planning decisions. Pharmacies should at least receive the amount they paid to purchase a drug.

***(And if not, [to the above question])*, What specific policies would do so?**

3. **Mr. Mitchell, in December, 2020 the U.S. Supreme Court unanimously ruled in *Rutledge v. PCMA* that ERISA does not preempt an Arkansas state law that regulates reimbursement levels paid by PBMs to pharmacies and that other states can follow suit. What are your thoughts on possible federal legislation, similar to Arkansas' state law, to ensure PBMs reimburse pharmacies at least at a price equal to the pharmacies' acquisition costs so that pharmacies can stay open for rural and underserved communities, to avoid pharmacy deserts where we need health care providers the most to ensure health equity in this country?**

See above.

[Questions submitted for the record and the responses by Mr. Holtz-Eakin follow:]

May 13, 2021

Mr. Douglas Holtz-Eakin, Ph.D.
President
American Action Forum
1747 Pennsylvania Avenue NW
Washington, D.C. 20006

Dear Dr. Holtz-Eakin:

I would like to thank you for testifying at the May 5, 2021 Subcommittee on Health, Employment, Labor, and Pensions hearing entitled "*Lower Drug Costs Now: Expanding Access to Affordable Health Care.*"

Please find enclosed additional questions submitted by Committee members following the hearing. Please provide a written response no later than Thursday, May 20, 2021, for inclusion in the official hearing record. Your responses should be sent to Mariah Mowbray and Daniel Foster of the Committee staff. They can be contacted at 202-225-3725 should you have any questions.

I appreciate your time and continued contribution to the work of the Committee.

Sincerely,

ROBERT C. "BOBBY" SCOTT
Chairman

Enclosure

Subcommittee on Health, Employment, Labor, and Pensions Hearing
"Lower Drug Costs Now: Expanding Access to Affordable Health Care"
Wednesday, May 5, 2021
12:00 p.m. (Eastern Time)

Ranking Member Virginia Foxx (R – NC)

1. Dr. Holtz-Eakin, in your testimony you state that prescription drug spending as a percent of national health expenditure has remained steady since 2000. Your testimony indicates that the cost of hospital care is the key element driving the growth in our country's national health expenditure. Do you believe that increasing the number of FDA approved or authorized prescription drugs can help lower the costs of hospital care in the United States? Are there any examples of how new drugs have lowered health expenditures?

Representative Diana Harshbarger (R – TN)

1. Dr. Holtz-Eakin, how do Pharmacy Benefit Managers (PBMs) impact patient costs and access to pharmacies?

2. Dr. Holtz-Eakin, how might reforms in the PBM industry and greater transparency itself lead to lowering prices, and perhaps level the playing field for independent community pharmacies?

(And if not, [to the above question]), What specific polices would do so?

3. Dr. Holtz-Eakin, in December, 2020 the U.S. Supreme Court unanimously ruled in *Rutledge v. PCMA* that ERISA does not preempt an Arkansas state law that regulates reimbursement levels paid by PBMs to pharmacies and that other states can follow suit. What are your thoughts on possible federal legislation, similar to Arkansas' state law, to ensure PBMs reimburse pharmacies at least at a price equal to the pharmacies' acquisition costs so that pharmacies can stay open for rural and underserved communities, to avoid pharmacy deserts where we need health care providers the most to ensure health equity in this country?

4. Dr. Holtz-Eakin, in your testimony you say we should realign the "perverse incentives", specifically around rebates, which drugmakers pay to PBMs to secure preferred status on a drug plan formulary. If the U.S. government were to follow your recommendations to realign incentives as proposed in your testimony, how soon might Americans see price relief at the pharmacy counter, and what would be the likely result for the current market players (taxpayers, health insurers and PBMs currently contracted to manage Medicare Part D benefits, etc.)?

Representative Scott Fitzgerald (R – WI)

1. Mr. Holtz-Eakin, the State of Wisconsin passed a law last month requiring pharmacy benefit managers to report to the state the rebates they receive from drug companies and whether or not those savings were passed along to the public. Do you think a nationwide law like this would lower drug prices? If so, how?

2. Mr. Holtz-Eakin, in response to the COVID-19 pandemic, President Trump began Operation Warp Speed. Through that partnership, the pharmaceutical industry developed lifesaving vaccines in record time. Would H.R. 3 stifle innovation like this and prevent this extraordinary effort from being duplicated in a future pandemic?

3. Mr. Holtz-Eakin, while there are a number of different ways to tackle rebate reform, it's clear to me that we need to do something to shed light on this opaque world. The Trump Administration took steps toward making changes to the status quo through the Anti-Kickback Statute, finalizing rulemaking last fall aimed at passing rebates and discounts on to patients. Do you support the concept of rebate reform in Medicare Part D? Do you think PBMs should be sharing any negotiated savings with patients to ensure that they benefit by paying less for drugs at the pharmacy counter?

Ranking Member Virginia Foxx (R – NC)

1. Dr. Holtz-Eakin, in your testimony you state that prescription drug spending as a percent of national health expenditure has remained steady since 2000. Your testimony indicates that the cost of hospital care is the key element driving the growth in our country's national health expenditure. Do you believe that increasing the number of FDA approved or authorized prescription drugs can help lower the costs of hospital care in the United States? Are there any examples of how new drugs have lowered health expenditures?

    - We have seen the benefits of increased drugs on the market putting downward pressure on the cost of hospital care since the early 20th century. The discovery of penicillin and antibiotics removed pneumonia, tuberculosis, and septicemia from the list of top causes of American deaths and made hospitals much safer. From a peak of hospital spending and a trough of pharmaceutical spending as a percentage of National Health Expenditures in 1980, a steady rise in pharmaceutical spending has correlated with a decrease in hospital spending. Increased spending on drugs that treat illnesses currently only treated in hospitals will inherently lead to less hospital treatments and thus lower spending on hospital care.

Representative Diana Harshbarger (R – TN)

1. Dr. Holtz-Eakin, how do Pharmacy Benefit Managers (PBMs) impact patient costs and access to pharmacies?

    - PBMs impact patient costs and access to pharmacies in a number of ways. In terms of access, PBMs establish pharmacy networks and charge fees to pharmacies to be a preferred network provider, potentially limiting the pharmacies a patient can use to fill prescriptions. These fees may also affect costs by being passed on to the patient by the pharmacy. PBMs also negotiate formularies with drug manufacturers and rebates—as a percent of the list price. These rebates can result in favoring drugs with a higher list price that may increase point-of-sale prices for individuals paying out-of-pocket for their prescriptions but can also be used to reduce overall premiums and cost-sharing for enrollees.

2. Dr. Holtz-Eakin, how might reforms in the PBM industry and greater transparency itself lead to lowering prices, and perhaps level the playing field for independent community pharmacies?

(And if not, [to the above question]), What specific polices would do so?

    - Greater transparency in PBMs may lead to price reductions, but that depends on the type of transparency. Independent community pharmacies have small market share and even if they knew what deals other pharmacies had with PBMs, it is unlikely the independent community pharmacies have the leverage to negotiate for better deals. Alternatively, large payers may benefit from such transparency since they have the

leverage to negotiate. The potential for cost-reduction through transparency depends on the context.

3. Dr. Holtz-Eakin, in December, 2020 the U.S. Supreme Court unanimously ruled in *Rutledge v. PCMA* that ERISA does not preempt an Arkansas state law that regulates reimbursement levels paid by PBMs to pharmacies and that other states can follow suit. What are your thoughts on possible federal legislation, similar to Arkansas' state law, to ensure PBMs reimburse pharmacies at least at a price equal to the pharmacies' acquisition costs so that pharmacies can stay open for rural and underserved communities, to avoid pharmacy deserts where we need health care providers the most to ensure health equity in this country?

    - Regulation at the federal level is fraught with unintended consequences, including a lack of flexibility based on the needs of a given state. Even at the state level, I do not think it is a good idea to allow governments to open up private contracts.

Representative Scott Fitzgerald (R – WI)

1. Mr. Holtz-Eakin, the State of Wisconsin passed a law last month requiring pharmacy benefit managers to report to the state the rebates they receive from drug companies and whether or not those savings were passed along to the public. Do you think a nationwide law like this would lower drug prices? If so, how?

    - A law like Wisconsin's may lower drug prices, but the effect would likely be minimal. Its benefit would come from payers having enough leverage over PBMs to act on the knowledge of the rebates PBMs receive to obtain a higher portion of the manufacturer's rebate and ostensibly pass it on to patients through premium decreases. PBMs are increasingly owned by insurers, so the value of the PBM's share of a rebate is often already known to the insurer and the rebate agreement is unlikely to change based on arrangements by other PBMs and payers. Simply knowing the rebate amount a PBM receives does not mean list prices for drugs will decrease; in some cases, it could lead to the creation of a price floor and prevent further savings.

2. Mr. Holtz-Eakin, in response to the COVID-19 pandemic, President Trump began Operation Warp Speed. Through that partnership, the pharmaceutical industry developed lifesaving vaccines in record time. Would H.R. 3 stifle innovation like this and prevent this extraordinary effort from being duplicated in a future pandemic?

    - Absolutely. H.R. 3 would lessen the ability of manufacturers to push out newer, more innovative drugs for complex conditions at a faster pace. The COVID-19 vaccine, while developed with heavy federal funding, was developed so quickly due to years of industry research and development prior to the pandemic. If H.R. 3 had been in place in the years before the pandemic such innovation would likely have been more difficult and slower, exacerbating the human and economic costs of the pandemic.

3. Mr. Holtz-Eakin, while there are a number of different ways to tackle rebate reform, it's clear to me that we need to do something to shed light on this opaque world. The Trump Administration took steps toward making changes to the status quo through the Anti-Kickback Statute, finalizing rulemaking last fall aimed at passing rebates and discounts on to patients. Do you support the concept of rebate reform in Medicare Part D? Do you think PBMs should be sharing any negotiated savings with patients to ensure that they benefit by paying less for drugs at the pharmacy counter?

   - Yes, rebate reform is very much needed in Part D. The true problem the American people have with prescription drug prices is that patient out-of-pocket costs are too high, which largely stems from payers competing for enrollees on the basis of low premiums. This means costs are shifted to patients at the pharmacy counter. Passing rebates directly on to beneficiaries and allowing beneficiaries to pay coinsurance on the net price rather than the list price, as is done with all other insurance-covered health services, would provide significant cost savings for the beneficiary. Premiums would only slightly rise for everyone, reflecting the true cost of care and spreading risk away from those who would be harmed by that risk the most.

[Questions submitted for the record and the responses by Mr. Isasi follow:]

May 13, 2021

Mr. Frederick Isasi, J.D., MPH
Executive Director
Families USA
1225 New York Avenue NW, Suite 800
Washington, D.C. 20005

Dear Mr. Isasi:

I would like to thank you for testifying at the May 5, 2021 Subcommittee on Health, Employment, Labor, and Pensions hearing entitled "*Lower Drug Costs Now: Expanding Access to Affordable Health Care.*"

Please find enclosed additional questions submitted by Committee members following the hearing. Please provide a written response no later than Thursday, May 20, 2021, for inclusion in the official hearing record. Your responses should be sent to Mariah Mowbray and Daniel Foster of the Committee staff. They can be contacted at 202-225-3725 should you have any questions.

I appreciate your time and continued contribution to the work of the Committee.

Sincerely,

ROBERT C. "BOBBY" SCOTT
Chairman

Enclosure

Subcommittee on Health, Employment, Labor, and Pensions Hearing
"Lower Drug Costs Now: Expanding Access to Affordable Health Care"
Wednesday, May 5, 2021
12:00 p.m. (Eastern Time)

Chairman Robert C. "Bobby" Scott (D – VA)

1. Mr. Isasi, what policy changes would you recommend Congress and this Committee consider to make employer-sponsored coverage more affordable for consumers?

Representative Diana Harshbarger (R – TN)

1. Mr. Isasi, how do Pharmacy Benefit Managers (PBMs) impact patient costs and access to pharmacies?

2. Mr. Isasi, how might reforms in the PBM industry and greater transparency itself lead to lowering prices, and perhaps level the playing field for independent community pharmacies?

(And if not, [to the above question]), What specific polices would do so?

3. Mr. Isasi, HRSA recently announced the New Reimbursement Program for COVID-19 Vaccine Administration Fees not Covered by Insurance | HHS.gov that will help to pay providers, including pharmacists, the $40 vaccination administration fee necessary to administer COVID-19 vaccines if health plans don't cover the vaccines or implement copays. However, I'm wondering does this open the door for private insurers and ERISA plans to pay pharmacists and other providers less for COVID-19 vaccine administration by imposing co-shares that it knows the federal government will pick up, which the pharmacist can't collect, and will only result in pharmacists and other vaccinators having to do more paperwork to get that co-share through this program? Should all plans, including ERISA plans, simply cover Medicare rates for COVID-19 vaccine administration?

4. Mr. Isasi, I am also hearing that many vaccinators, including pharmacies, are facing increased routine audits from PBMs regarding COVID-19 vaccinations. Is now really the right time for PBMs to audit services that are literally saving lives? (Especially when it has been widely reported that PBMs have engaged in abusive audit practices and penalized pharmacies for minor, typographical errors on claims, forcing them to forego reimbursement due to small errors that posed no consequence to the claim.) Would you support federal legislation or regulatory action suspending routine PBM audits for COVID-19 services until we have defeated the current pandemic and during future health crises?

House Committee on Education and Labor
Subcommittee on Health, Employment, Labor, and Pensions Hearing
"Lower Drug Costs Now: Expanding Access to Affordable Health Care"
Wednesday, May 5, 2021, 12:00 p.m. (Eastern Time)
Questions for the Record

Chairman Robert C. "Bobby" Scott (D – VA)

1. **Mr. Isasi, what policy changes would you recommend Congress and this Committee consider to make employer-sponsored coverage more affordable for consumers?**

The vast majority of American families below retirement age receive their health coverage through employer-sponsored insurance (ESI), and this Committee has both the jurisdiction and the mandate to improve that coverage. Rising health care costs stemming from decades of egregious prescription drug price increases and staggeringly high-launch prices, in addition to the rising trends of horizontal and vertical health care industry consolidation, have made ESI much less affordable for both employers and employees and their families. Families most often feel this pain in the forms of rising premiums and out-of-pocket costs for prescription drugs. The total cost of an employer-sponsored health insurance plan for one family grew from $5,791 in 1999 to $21,342 in 2020.[1]

Our nation's small businesses are hit hard by this rapid cost increase and are struggling to afford healthcare benefits for their employees. Recent polling of small businesses shows nine out of ten employers report that drug prices are among the greatest threats to affordability of health coverage for their employees.[2] Likewise, large employers and their employees are impacted by rising health care costs. A recent survey of executive decision makers at over 300 large private employers showed nearly unanimous agreement that health benefit costs are excessive, and 86% of respondents viewed a greater government role in providing coverage and containing health care costs to be beneficial to their employees.[3]

Moreover, as families and employers try to lower premium costs, more and more families receive lower quality coverage. In particular, the rate of underinsurance, meaning the rate of insured people who have unaffordable cost-sharing requirements, has increased by almost 50 percent since 2010.[4]

Policymakers should focus on both short- and long-term solutions that can expand access to high quality health coverage and lower health care costs.

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[1] Gary Claxton et al., Employer Health Benefits: 2020 Annual Survey (San Francisco, CA: Kaiser Family Foundation, October 2020), https://www.kff.org/report-section/ehbs-2020-summaryof-findings/
[2] https://smallbusinessmajority.org/our-research/healthcare/small-businesses-struggling-access-healthcare-during-covid-19-pandemic
[3] https://www.pbgh.org/wp-content/uploads/2021/04/9704-How-Corporate-Executives-View-Rising-Health-Care-Costs-and-the-Role-of-Government-v2.pdf
[4] Sara Collins et. al., Insurance Coverage in 2020: A Looming Crisis in Affordability: Findings from the Commonwealth Fund Biennial Health Insurance Survey, 2020 (Washington D.C., Commonwealth Fund), https://www.commonwealthfund.org/sites/default/files/2020-08/Collins_looming_crisis_affordability_biennial_2020_sb.pdf

To take a critical step in addressing prescription drug costs and improving affordability for both employers and consumers, Congress and the Committee should advance H.R. 3, which:

- Authorizes and mandates that the Secretary of Health and Human Services negotiate directly with drug manufacturers on insulin and at least 50 other drugs that lack competition with the greatest costs to Medicare and the U.S. health system.
- Establishes a maximum negotiated price of no more than 1.2 times the average price offered in six other countries (Australia, Canada, France, Germany, Japan, and the United Kingdom).
- Requires manufacturers to make the negotiated price available to other purchasers.
- Provides a strong incentive for manufacturers to negotiate in good faith and to provide the negotiated price to Medicare and other purchasers through the use of an escalating excise tax and civil monetary penalties.
- Limits manufacturers' ability to hike the price of drugs year after year by imposing inflation rebates in Medicare Parts B and D.
- Caps out-of-pocket spending for seniors in Part D at $2000.

The Committee should further ensure that employers and employees don't bear the brunt of pharmaceutical industry price gouging by enacting strong incentives and/or penalties to ensure that manufacturers cannot raise prices above the rate of inflation for non-Medicare purchasers as well. This is particularly critical for drugs which have a relatively low exposure to Medicare – such as pediatric drugs.

Additionally, policymakers should prioritize legislation to end patent abuses and require price transparency throughout the pharmaceutical supply and financing chains, and ensure that consumers have affordable access to approved drugs that have been developed through significant public investment by preventing manufacturers from being awarded exclusivities that allow for price gouging.

To take steps to address health care costs more broadly, Congress and the Committee should work to:

- **Improve affordability of individual-market coverage.**
  - Make permanent the provisions of the American Rescue Plan (ARP) that cap premium costs for people with incomes above 400% of the federal poverty level (FPL) and increase financial assistance for people at lower income levels.
  - Substantially reduce deductibles and other out-of-pocket costs by extending and expanding cost-sharing reductions (CSRs) for consumers with incomes up to 400% of FPL.
  - With the above affordability improvements assured, Congress could also take both of the following steps: change the base for advance premium tax credits (APTCs) from silver to gold levels, improving affordability for consumers with incomes above 400% FPL, and restore federal CSR payments to insurers.
- **Improve immigrant eligibility**. End artificial barriers to immigrant eligibility by permitting all lawfully present individuals to qualify for insurance affordability programs (IAPs) – Medicaid, the Children's Health Insurance Program (CHIP), the Basic Health Program (BHP), advance premium tax credits (APTCs), and cost-sharing reductions (CSRs).

- **Increase APTCs for younger adults and extend APTCs for other specific populations.**
  - Fix the "family glitch" that currently denies APTCs based on unaffordable employer offers of dependent coverage, without exposing employers to additional liability.
  - Let APTCs pay for student health plans that fully comply with the Affordable Care Act (ACA).
- **Help with ESI out-of-pocket costs**. For people who are ineligible for APTCs because of ESI coverage offers and who either have low incomes or spend a very large percentage of their income on health care, provide federal financial assistance to help pay the worker's share of out-of-pocket costs.
- **Address social drivers of health (SDOH) in private coverage**. Clarify that health plans, including employer-sponsored and multi-employer plans, may cover carefully-defined services, distributed equitably and focused on vulnerable workers, to address SDOH without violating the Employee Retirement Income Security Act of 1974 (ERISA) or the Internal Revenue Code's rules for tax-excluded health benefits. Examples include paying for services to address food insecurity, housing instability, and domestic violence.
- **Provide federal financial support to improve affordability or availability of ESI.**
  - Provide tax credits or other financial assistance for small-group coverage, with extra help for the smallest firms and the self-employed, and targeting based on employee income.
  - Reduce overall premium costs by providing reinsurance to ESI plans for extremely high-cost consumers outside the normal scope of stop-loss coverage.
- **Address underlying industry abuses and market failures that drive costs into the overall system.**
  - Address consolidated health care markets.
    - Prohibit anti-competitive terms in provider and insurer contracts that limit access to higher-quality, lower-cost care.
    - Mandate site-neutral payments requiring Medicare and Medicaid to pay the same rates across hospital outpatient departments (on and off campus), ambulatory surgery centers, freestanding and non-freestanding emergency departments, and off-campus physician offices, while protecting access to care in underserved rural and urban communities.
  - Improve data collection and transparency in pricing, costs, and quality.
    - Establish a national all-payer claims database (APCD) and require all payers, providers, and public health agencies to participate in mandatory exchange of accurate, real-time data across medical, clinical, prescription drug, dental, behavioral health, and social services, and expanding interoperability standards to support this exchange of data.
    - Require payers and providers to disclose negotiated rates.
    - Establish harmonized reporting of performance measures by providers across all payers, including a core set of disparity reduction measures. Data should be stratified by age, sex, race, ethnicity, and primary language, at a minimum, and be extended to other demographic factors, such as socioeconomic status, gender identity, sexual orientation, and disability, as data are collected and become available

Representative Diana Harshbarger (R – TN)

1. Mr. Isasi, how do Pharmacy Benefit Managers (PBMs) impact patient costs and access to pharmacies?

As discussed below, reforming PBMs is an important component of addressing prescription drug pricing abuses. However, the most important factor driving up pharmaceutical prices is the price being charged by manufactures. So, any meaningful reform aimed at making prescription drugs affordable should directly address launch prices and price increases by the pharmaceutical industry.

All transactions along the pharmaceutical supply and finance chains - including with PBMs - are dependent upon the list price of the drug. Pharmaceutical prices are established at the launch of the drug (during the patent exclusivity period), and many experts note that the higher-than-competitive prices are set during this time to maximize profits and are "constrained only by how much consumers are willing to pay for a product that is protected by exclusivity" (i.e. what the market will bear).[i,ii] During the exclusivity period, competition is thwarted and PBMs and other payers and purchasers have virtually no leverage to negotiate prices for these sole source drugs; and therefore, have limited impact on the ultimate cost to patients.

As I noted in my testimony, Congress created a system in which drug companies and their lawyers are incentivized to ensure these exclusivity periods last as long as possible instead of rewarding the innovative, life-changing drugs we all want and need. For example, the top 12 best-selling drugs in the United States in 2017 averaged 125 patent applications per drug, resulting in an average 38 years of attempted blocked competition, and they increased in price significantly despite being on the market for 15 years.[iii]

To be sure, the pharmaceutical supply and financing chains include a number of interdependent intermediaries, including wholesalers, health insurance plans, PBMs, pharmacies (retail, specialty, and independent), all generating profits and ultimately affecting affordability of medicines to consumers. Experts find that the complexity and opacity of the supply chain with its multiple market segments and plethora of incentives and trade-offs makes it extremely difficult to understand the role of a single player.[iv] PBMs, hospitals and specialty clinics operate with large margins and receive discounts and rebates in the context of the perverse incentives in place due to the broken market that Congress created.[v] Only a small portion of these savings make it to consumers, and those most at risk of financial burden pay the most (i.e. the uninsured).[vi]

Sensible PBM reforms can bring transparency to this opaque market and are part of a comprehensive strategy to ensure that affordable drugs make it to consumers. But it all starts with the price. Upending the perverse pricing scheme hinges on a federal legislative agenda that incentivizes manufacturers to innovate, not block competition, creating healthy market dynamics. Nearly 9 out of 10 consumers agree; across political affiliation, voters want Congress to level the playing field by allowing Medicare to negotiate drug prices and reining in abusive price increases.[vii]

2. Mr. Isasi, how might reforms in the PBM industry and greater transparency itself lead to lowering prices, and perhaps level the playing field for independent community pharmacies?

It is important to reiterate the points made above: reforming PBMs is an important component of addressing prescription drug pricing abuses, but the most important factor driving up pharmaceutical prices is the price being charged by manufacturers. So, any meaningful reform aimed at making prescription drugs affordable should directly address launch prices and price increases by the pharmaceutical industry.

In recent years, states have taken the lead in regulating the PBM industry by increasing transparency, oversight and accountability through registration and licensure, and addressing payment and auditing practices to reduce the ***cost burden*** on consumers and independent pharmacies.[viii] States have passed laws that require pass-through of rebates, ensuring pharmacists can share the lowest out-of-pocket option at the counter, and elimination of "spread pricing." Contractual oversight has tightened and state employee programs have innovative PBM procurement processes to achieve savings.[ix] Examples include New Jersey's reverse auction approach, which projects billions in savings,[x] and Wisconsin Employee Trust Fund's full pass-through contracting model in which all rebates and discounts are retained by the Trust Fund to lower costs for members.

However, given the market distortions that lead to high and rising drug prices and endless monopolies that thwart competition, the impact on ***prices*** for these types of policies are limited. In fact, a recent analysis shows the rise in drug prices (per unit) over a 12-year study period (2006 to 2017) far outstripped the effect of rebates; post-rebate prices rose a stunning 313 percent.[xi] During the same period, the gap between brand and generic drug prices widened significantly, showing that generics also are not reducing drug prices as they are intended to do.

With respect to lowering drug ***prices***, the playing field can only be leveled through Congressional action. Congress must address the market distortions created by current law. Eliminating the ban on Medicare negotiation so that America's families no longer have to pay three times more than their peers in other developed countries,[xii] and reining in abusive price increases by penalizing manufacturers whose drug prices grow faster than the rate of inflation. In the long run, Congress must address the root cause of an ineffective market through policies that incentivize innovation and disincentivize extended monopolies on old, and often low value drugs.

3. Mr. Isasi, HRSA recently announced the New Reimbursement Program for COVID-19 Vaccine Administration Fees not Covered by Insurance | HHS.gov that will help to pay providers, including pharmacists, the $40 vaccination administration fee necessary to administer COVID-19 vaccines if health plans don't cover the vaccines or implement copays. However, I'm wondering does this open the door for private insurers and ERISA plans to pay pharmacists and other providers less for COVID-19 vaccine administration by imposing co-shares that it knows the federal government will pick up, which the pharmacist can't collect, and will only result in pharmacists and other vaccinators having to do more paperwork to get that co-share through this program? Should all plans, including ERISA plans, simply cover Medicare rates for COVID-19 vaccine administration?

COVID-19 vaccines should be free (including without cost-sharing requirements) and easily accessible to all people in the United States. In particular, trusted providers and independent pharmacists in underserved areas play a vital role in ensuring access and engaging hesitant individuals in education about vaccines. Persistently inequitable vaccination rates in communities of

color, despite clear demand,[xiii] is of particular concern, and we encourage Congress to identify and enact policies that limit barriers to access, eliminate inequities, and incentivize trusted sources to engage robustly in the public health engagement process.

4. **Mr. Isasi, I am also hearing that many vaccinators, including pharmacies, are facing increased routine audits from PBMs regarding COVID-19 vaccinations. Is now really the right time for PBMs to audit services that are literally saving lives? (Especially when it has been widely reported that PBMs have engaged in abusive audit practices and penalized pharmacies for minor, typographical errors on claims, forcing them to forego reimbursement due to small errors that posed no consequence to the claim.) Would you support federal legislation or regulatory action suspending routine PBM audits for COVID-19 services until we have defeated the current pandemic and during future health crises?**

Pharmacists are playing an essential role in COVID-19 vaccinations. Streamlining routine oversight practices and workflows while ensuring consumer safety is paramount during a health crisis such as the COVID-19 pandemic. Families USA supports striking that balance. Federal and state public health emergency declarations have allowed new flexibilities in other parts of the health system and could be considered (where applicable in state law and emergency authority).

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[xii] Andrew W. Mulcahy et al., *International Prescription Drug Price Comparison: Current Empirical Estimates and Comparisons with Previous Studies* (Santa Monica, CA: Rand Corporation, 2021), https://www.rand.org/pubs/research_reports/RR2956.html

[xiii] Kaiser Family Foundation. "Vaccine Monitor: Unvaccinated Hispanic Adults are Twice as Likely as White Adults to Want a COVID-19 Vaccine ASAP, Highlighting a Key Outreach Opportunity for Vaccination Efforts." May 13 2021. https://www.kff.org/other/press-release/vaccine-monitor-unvaccinated-hispanic-adults-are-twice-as-likely-as-white-adults-to-want-a-covid-19-vaccine-asap-highlighting-a-key-outreach-opportunity-for-vaccination-efforts/

[Whereupon, at 2:15 p.m., the Subcommittee was adjourned.]

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